PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12N 15/12, C12P 21/02 A61K 37/02, C12N 5/12 C07K 15/06	A1	(11) International Publication Number: WO 93/09229
		(43) International Publication Date: 13 May 1993 (13.05.93)

(21) International Application Number: PCT/US92/09430

(22) International Filing Date: 2 November 1992 (02.11.92)

(30) Priority data:

787,496	4 November 1991 (04.11.91)	US
864,692	7 April 1992 (07.04.92)	US

(81) Designated States: AU, BR, CA, FI, HU, JP, KR, NO, RU, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).

Published
With international search report.

(71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 Cambridge Park Drive, Cambridge, MA 02140 (US).

(72) Inventors: ISRAEL, David ; 117 Anson Road, Concord, MA 01742 (US). WOLFMAN, Neil, M. ; 30 Rolling Lane, Dover, MA 02030 (US).

(74) Agent: KAPINOS, Ellen, J.; Genetics Institute, Inc., 87 Cambridge Park Drive, Cambridge, MA 02140 (US).

(54) Title: RECOMBINANT BONE MORPHOGENETIC PROTEIN HETERODIMERS, COMPOSITIONS AND METHODS OF USE

(57) Abstract

The present invention relates to methods for producing recombinant heterodimeric BMP proteins useful in the field of treating bone defects, healing bone injury and in wound healing in general. The invention also relates to the recombinant heterodimers and compositions containing them.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SK	Slovak Republic
SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
UA	Ukraine
US	United States of America
VN	Viet Nam

RECOMBINANT BONE MORPHOGENETIC PROTEIN HETERODIMERS, COMPOSITIONS AND METHODS OF USE

Field of the Invention

The present invention relates to a series of novel recombinant heterodimeric proteins useful in the field of treating bone defects, healing bone injury and in wound healing in general. The invention also relates to methods for obtaining these heterodimers, methods for producing them by recombinant genetic engineering techniques, and compositions containing them.

Background of the Invention

In recent years, protein factors which are characterized by bone or cartilage growth inducing properties have been isolated and identified. See, e.g., U. S. Patent No. 5,013,649, PCT published application WO90/11366; PCT published application WO91/05802 and the variety of references cited therein. See, also, PCT/US90/05903 which discloses a protein sequence termed OP-1, which is substantially similar to human BMP-7, and has been reported to have osteogenic activity.

A family of individual bone morphogenetic proteins (BMPs), termed BMP-2 through BMP-9 have been isolated and identified. Incorporated by reference for the purposes of providing disclosure of these proteins

and methods of producing them are co-owned, co-pending U. S. Patent Application SN 721,847 and the related applications recited in its preamble. Of particular interest, are the proteins termed BMP-2 and BMP-4, disclosed in the above-referenced application; BMP-7, disclosed in SN 438,919; BMP-5, disclosed in SN 370,547 and SN 356,033; and BMP-6, disclosed in SN 370,544 and SN 347,559; and BMP-8, disclosed in SN 525,357. Additional members of the BMP family include BMP-1, disclosed in SN 655,578; BMP-9, disclosed in SN 720,590; and BMP-3, disclosed in SN 179,197 and PCT publication 89/01464. These applications are incorporated herein by reference for disclosure of these BMPs.

There remains a need in the art for other proteins and compositions useful in the fields of bone and wound healing.

Summary of the Invention

In one aspect, the invention provides a method for producing a recombinant heterodimeric protein having bone stimulating activity comprising culturing a selected host cell containing a polynucleotide sequence encoding a first selected BMP or fragment thereof and a polynucleotide sequence encoding a second selected BMP or fragment thereof. The resulting co-expressed, biologically active heterodimer is isolated from the culture medium.

According to one embodiment of this invention,

the host cell may be co-transfected with one or more vectors containing coding sequences for one or more BMPs. Each BMP polynucleotide sequence may be present on the same vector or on individual vectors transfected into the cell. Alternatively, the BMPs or their fragments may be incorporated into a chromosome of the host cell. Additionally, a single transcription unit may encode single copy of two genes encoding a different BMP.

According to another embodiment of this invention, the selected host cell containing the two polypeptide encoding sequences is a hybrid cell line obtained by fusing two selected, stable host cells, each host cell transfected with, and capable of stably expressing, a polynucleotide sequence encoding a selected first or second BMP or fragment thereof.

In another aspect of the present invention, therefore, there are provided recombinant heterodimeric proteins comprising a protein or fragment of a first BMP in association with a protein or fragment of a second BMP. The heterodimer may be characterized by bone stimulating activity. The heterodimers may comprise a protein or fragment of BMP-2 associated with a protein or fragment of either BMP-5, BMP-6, BMP-7 or BMP-8; or a protein or fragment of BMP-4 associated with a protein or fragment of either BMP-5, BMP-6, BMP-7 or BMP-8. In further embodiments the heterodimers may comprise a protein or fragment of BMP-2 associated with a protein or

fragment of either BMP-1, BMP-3 or BMP-4. BMP-4 may also form a heterodimer in association with BMP-1, BMP-2 or a fragment thereof. Still further embodiments may comprise heterodimers involving combinations of BMP-5, BMP-6, BMP-7 and BMP-8. For example, the heterodimers may comprise BMP-5 associated with BMP-6, BMP-7 or BMP-8; BMP-6 associated with BMP-7 or BMP-8; or BMP-7 associated with BMP-8. These heterodimers may be produced by co-expressing each protein in a selected host cell and isolating the heterodimer from the culture medium.

As a further aspect of this invention a cell line is provided which comprises a first polynucleotide sequence encoding a first BMP or fragment thereof and a second polynucleotide sequence encoding a second BMP or fragment thereof, the sequences being under control of one or more suitable expression regulatory systems capable of co-expressing the BMPs as a heterodimer. The cell line may be transfected with one or more than one polynucleotide molecule. Alternatively, the cell line may be a hybrid cell line created by cell fusion as described above.

Another aspect of the invention is a polynucleotide molecule or plasmid vector comprising a polynucleotide sequence encoding a first selected BMP or fragment thereof and a polynucleotide sequence encoding a second selected BMP or fragment thereof. The sequences are under the control of at least one suitable regulatory

sequence capable of directing co-expression of each protein or fragment. The molecule may contain a single transcription unit containing a copy of both genes, or more than one transcription unit, each containing a copy of a single gene.

As still another aspect of this invention there is provided a method for producing a recombinant dimeric or heterodimeric protein having bone stimulating activity in a prokaryotic cell comprising culturing a selected host cell containing a polynucleotide sequence encoding a first selected BMP or fragment thereof; culturing a second selected host cell containing a polynucleotide sequence encoding a second selected BMP or fragment thereof; isolating monomeric forms of each BMP protein from the culture medium and co-assembling a monomer of the first protein with a monomer of the second protein. The first protein and the second protein may be the same or different BMPs. The resulting biologically active dimer or heterodimer is thereafter isolated from the mixture. Preferred cells are E. coli.

Thus, as further aspects of this invention recombinant BMP dimers or heterodimers produced in eukaryotic cells are provided, as well as suitable vectors or plasmids, and selected transformed cells useful in such a production method.

Other aspects and advantages of the present invention are described further in the following detailed

description of preferred embodiments of the present invention.

Brief Description of the Figures

Figure 1 provides the DNA and amino acid sequences of human BMP-2 (SEQ ID NOs: 1 and 2).

Figure 2 provides the DNA and amino acid sequences of human BMP-4 (SEQ ID NOs: 3 and 4).

Figure 3 provides the DNA and amino acid sequences of human BMP-7 (SEQ ID NOs: 5 and 6).

Figure 4 provides the DNA and amino acid sequences of human BMP-6 (SEQ ID NOs: 7 and 8).

Figure 5 provides the DNA and amino acid sequences of human BMP-5 (SEQ ID NOs: 9 and 10).

Figure 6 provides the DNA and amino acid sequences of human BMP-8 (SEQ ID NOs: 11 and 12).

Figure 7 provides the DNA sequence of vector pALB2-781 containing the mature portoin of the BMP-2 gene (SEQ ID NOs: 13 and 14).

Figure 8 compares the activity of CHO BMP-2 and CHO BMP-2/7 in the W20 alkaline phosphatase assay.

Figure 9 compares the activity of CHO BMP-2 and CHO BMP-2/7 in the BGP (osteocalcin) assay.

Figure 10 provides a comparison of the W-20 activity of E. coli produced BMP-2 and BMP-2/7 heterodimer.

Figure 11 depicts BMP-3 DNA and amino acid sequence.

Figure 12 provides a comparison of BMP-2 and BMP-2/6

in the W-20 assay.

Figure 13 provides a comparison of the in vivo activity of BMP-2/6 and BMP-2.

Figure 14 provides a comparison of BMP-2, BMP-6 and BMP-2/6 in vivo activity.

Detailed Description of the Invention

The present invention provides a method for producing recombinant heterodimeric proteins having bone stimulating activity, as well as the recombinant heterodimers themselves, and compositions containing them for bone-stimulating or repairing therapeutic use.

As used throughout this document, the term 'heterodimer' is defined as a biologically-active protein construct comprising the association of two different BMP protein monomers or active fragments thereof joined through at least one covalent, disulfide linkage. A heterodimer of this invention may be characterized by the presence of between one to seven disulfide linkages between the two BMP component strands.

According to the present invention, therefore, a method for producing a recombinant BMP heterodimer according to this invention comprises culturing a selected host cell containing a polynucleotide sequence encoding a first selected BMP or a biologically active fragment thereof and a polynucleotide sequence encoding a second selected BMP or a fragment thereof. The resulting

co-expressed, biologically active heterodimer is formed within the host cell, secreted therefrom and isolated from the culture medium. Preferred embodiments of methods for producing the heterodimeric proteins of this invention, are described in detail below and in the following examples. Preferred methods of the invention involve known recombinant genetic engineering techniques [See, e.g., Sambrook et al, "Molecular Cloning. A Laboratory Manual:", 2d edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)]. However, other methods, such as conventional chemical synthesis may also be useful in preparing a heterodimer of this invention.

BMP heterodimers generated by this method are produced in a mixture of homodimers and heterodimers. This mixture of heterodimers and homodimers may be separated from contaminants in the culture medium by resort to essentially conventional methods, such as classical protein biochemistry or affinity antibody columns specific for one of the BMPs making up the heterodimer. Additionally, if desired, the heterodimers may be separated from homodimers in the mixture. Such separation techniques allow unambiguous determination of the activity of the heterodimeric species. Example 4 provides one presently employed purification scheme for this purpose.

Preferably the recombinant heterodimers of this

invention produced by these methods involve the BMPs designated human BMP-2, human BMP-4, human BMP-5, human BMP-6, human BMP-7 and BMP-8. However, BMP-3 has also been determined to form an active heterodimer with BMP-2. Other species of these BMPs as well as BMPs than those specifically identified above may also be employed in heterodimers useful for veterinary, diagnostic or research use. However, the human proteins, specifically those proteins identified below, are preferred for human pharmaceutical uses.

Human BMP-2 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 1. Human BMP-2 proteins are further characterized as disulfide-linked dimers and homodimers of mature BMP-2 subunits. Recombinantly-expressed BMP-2 subunits include protein species having heterogeneous amino termini. One BMP-2 subunit is characterized by comprising amino acid #249 (Ser) - #396 (Arg) of Figure 1 (SEQ ID NOs: 1 and 2). Another BMP-2 subunit is characterized by comprising amino acid #266 (Thr) - #396 (Arg) of Figure 1. Another BMP-2 subunit is characterized by comprising amino acid #296 (Cys) - #396 (Arg) of Figure 1. A mature BMP-2 subunit is characterized by comprising amino acid #283 (Gln) - #396 (Arg) of Figure 1. This latter subunit is the presently most abundant protein species which results from recombinant expression of BMP-2 (Figure 1).

However, the proportions of certain species of BMP-2 produced may be altered by manipulating the culture conditions. BMP-2 may also include modifications of the sequences of Figure 1, e.g., deletion of amino acids #241-280 and changing amino acid #245 Arg to Ile, among other changes.

As described in detail in United States Patent Application SN 721,847, incorporated by reference herein, human BMP-2 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #356 to #1543 in Figure 1 and recovering and purifying from the culture medium one or more of the above-identified protein species, substantially free from other proteinaceous materials with which it is co-produced. Human BMP-2 proteins are characterized by the ability to induce bone formation. Human BMP-2 also has <u>in vitro</u> activity in the W20 bioassay. Human BMP-2 is further characterized by the ability to induce cartilage formation. Human BMP-2 may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay described in the above-referenced application.

Human BMP-4 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 2 (SEQ ID NOs: 3 and 4). Human BMP-4 proteins are

further characterized as disulfide-linked dimers and homodimers of mature BMP-4 subunits. Recombinantly-expressed BMP-4 subunits may include protein species having heterogeneous amino termini. A mature subunit of human BMP-4 is characterized by an amino acid sequence comprising amino acids #293 (Ser) - #408 (Arg) of Figure 2. Other amino termini of BMP-4 may be selected from the sequence of Figure 2. Modified versions of BMP-4, including proteins further truncated at the amino or carboxy termini, may also be constructed by resort to conventional mutagenic techniques.

As disclosed in above-incorporated patent application SN 721,847, BMP-4 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #403 to nucleotide #1626 in Figure 2 and recovering and purifying from the culture medium a protein containing the amino acid sequence from amino acid #293 to #408 as shown in Figure 2, substantially free from other proteinaceous materials with which it is co-produced. BMP-4 proteins are capable of inducing the formation of bone. BMP-4 proteins are capable of inducing formation of cartilage. BMP-4 proteins are further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay.

Human BMP-7 is characterized by containing substantially the entire sequence, or fragments, of the

amino acid sequence and DNA sequence disclosed in Figure 3. Human BMP-7 proteins are further characterized as disulfide-linked dimers and homodimers of mature BMP-7 subunits. Recombinantly-expressed BMP-7 subunits include protein species having heterogeneous amino termini. One BMP-7 subunit is characterized by comprising amino acid #293 (Ser) - #431 (His) of Figure 3 (SEQ ID NOs: 5 and 6). This subunit is the most abundantly formed protein produced by recombinant expression of the BMP-7 sequence. Another BMP-7 subunit is characterized by comprising amino acids #300 (Ser) - #431 (His) of Figure 3. Still another BMP-7 subunit is characterized by comprising amino acids #316 (Ala) - #431 (His) of Figure 3. Other amino termini of BMP-7 may be selected from the sequence of Figure 3. Similarly, modified versions, including proteins further truncated at the amino or carboxy termini, of BMP-7 may also be constructed by resort to conventional mutagenic techniques.

As disclosed in above-incorporated patent application SN 438,919, BMP-7 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #97 to nucleotide #1389 in Figure 3 and recovering and purifying from the culture medium a protein containing the amino acid sequence from amino acid #293 to #431 as shown in Figure 3, substantially free from other proteinaceous or contaminating materials with which it is

co-produced. These proteins are capable of stimulating, promoting, or otherwise inducing cartilage and/or bone formation.

Human BMP-6 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 4. Human BMP-6 proteins are further characterized as disulfide-linked dimers of mature BMP-6 subunits. Recombinantly-expressed BMP-6 subunits may include protein species having heterogeneous amino termini. One BMP-6 subunit is characterized by comprising amino acid #375 (Ser) - #513 (His) of Figure 4 (SEQ ID NOs: 7 and 8). Other amino termini of BMP-6 may be selected from the sequence of Figure 4. Modified versions, including proteins further truncated at the amino or carboxy termini, of BMP-6 may also be constructed by resort to conventional mutagenic techniques.

As described in detail in United States Patent Application SN 490,033, incorporated by reference herein, human BMP-6 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #160 to #1698 in Figure 4 and recovering and purifying from the culture medium a protein comprising amino acid #375 to #513 of Figure 4, substantially free from other proteinaceous materials or other contaminating materials with which it is co-produced. Human BMP-6 may be further characterized by

the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay.

Human BMP-5 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 5 (SEQ ID NOs: 9 and 10). Human BMP-5 proteins are further characterized as disulfide-linked dimers of mature BMP-5 subunits. Recombinantly-expressed BMP-5 subunits may include protein species having heterogeneous amino termini. One BMP-5 subunit is characterized by comprising amino acid #329 (Ser) - #454 (His) of Figure 5. Other amino termini of BMP-5 may be selected from the sequence of Figure 5. Modified versions, including proteins further truncated at the amino or carboxy termini, of BMP-5 may also be constructed by resort to conventional mutagenic techniques.

As described in detail in United States Patent Application SN 588,227, incorporated by reference herein, human BMP-5 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #701 to #2060 in Figure 5 and recovering and purifying from the culture medium a protein comprising amino acid #329 to #454 of Figure 5, substantially free from other proteinaceous materials or other contaminating materials with which it is co-produced. Human BMP-5 may be further characterized by the ability to demonstrate cartilage and/or bone

formation activity in the rat bone formation assay described in the above-referenced application.

Human BMP-8 is characterized by containing substantially the entire sequence, or fragments, of the amino acid sequence and DNA sequence disclosed in Figure 6. Human BMP-8 proteins may be further characterized as disulfide-linked dimers of mature BMP-8 subunits. Recombinantly-expressed BMP-8 subunits may include protein species having heterogeneous amino termini. A BMP-8 sequence or subunit sequence comprises amino acid #143 (Ala) - #281 (His) of Figure 6 (SEQ ID NOs: 11 and 12). Other amino termini of BMP-8 may be selected from the sequence of Figure 6. Modified versions, including proteins further truncated at the amino or carboxy termini, of BMP-8 may also be constructed by resort to conventional mutagenic techniques.

As described generally in United States Patent Application SN 525,357, incorporated by reference herein, and as further described herein, human BMP-8 may be produced by culturing a cell transformed with a DNA sequence comprising the nucleotide coding sequence from nucleotide #1 to #850 in Figure 6 and recovering and purifying from the culture medium a protein comprising amino acid #143 to #281 of Figure 6, or similar amino acid sequences with heterogenous N-termini, substantially free from other proteinaceous materials or other contaminating materials with which it is co-produced.

This BMP-8 may also be produced in E. coli by inserting into a vector the sequence encoding amino acid #143 to 281 of Figure 6 with a Met inserted before amino acid #143. Human BMP-8 may be further characterized by the ability to demonstrate cartilage and/or bone formation activity in the rat bone formation assay.

Each above described BMP protein in its native, non-reduced dimeric form may be further characterized by an apparent molecular weight on a 12% Laemmli gel ranging between approximately 28kD to approximately 40kD. Analogs or modified versions of the DNA and amino acid sequences described herein which provide proteins or active fragments displaying bone stimulating or repairing activity in the rat bone formation assay described below in Example 9, are also classifed as suitable BMPs for use in this invention, further provided that the proteins or fragments contain one or more Cys residues for participation in disulfide linkages. Useful modifications of these sequences may be made by one of skill in the art with resort to known recombinant genetic engineering techniques. Production of these BMP sequences in mammalian cells produces homodimers, generally mixtures of homodimers having heterologous N termini. Production of these BMP sequences in E.coli produces monomeric protein species.

Thus, according to this invention one recombinant heterodimer of the present invention

comprises the association of a human BMP-2, including, e.g., a monomeric strand from a mature BMP-2 subunit as described above or an active fragment thereof, bound through one or up to seven covalent, disulfide linkages to a human BMP-5 including, e.g., a monomeric strand from a mature BMP-5 subunit as described above or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-2, as described above, bound through one or up to seven covalent, disulfide linkages to a human BMP-6, including, e.g., a monomeric strand from a BMP-6 subunit as described above or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-2, as described above, bound through one or up to seven covalent, disulfide linkages to a human BMP-7, including, e.g., a monomeric strand of a BMP-7 subunit as described above or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-2, as described above, bound through one or up to seven covalent, disulfide linkages to a human BMP-8, including, e.g., a monomeric strand of a BMP-8 subunit as described above or an active fragment thereof.

Still another recombinant heterodimer of the present invention comprises the association of a human BMP-4, including, e.g., a monomeric strand of a BMP-4

subunit as described above or an active fragment thereof, bound through one or up to seven covalent, disulfide linkages to a human BMP-5, as described above. Another recombinant heterodimer of the present invention comprises the association of a human BMP-4, as described above, bound through one or more covalent, disulfide linkages to a human BMP-6, as described above. Another recombinant heterodimer of the present invention comprises the association of a human BMP-4, as described above bound through one or more covalent, disulfide linkages to a human BMP-7, as described above. Another recombinant heterodimer of the present invention comprises the association of a human BMP-4, as described above, bound through one or more covalent, disulfide linkages to a human BMP-8, as described above.

A further recombinant heterodimer of the present invention comprises the association of a human BMP-2, including, e.g., a monomeric strand from a mature BMP-2 subunit as described above or an active fragment thereof, bound through at least one disulfide linkage to a human BMP-3 including, e.g., a monomeric strand from a mature BMP-3 subunit as described above or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-2, as described above, bound through at least one disulfide linkage to a human BMP-4, including, e.g., a monomeric strand from a BMP-4 subunit as described above

or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-5, as described above, bound through at least one disulfide linkage to a human BMP-6, including, e.g., a monomeric strand of a BMP-6 subunit as described above or an active fragment thereof. Another recombinant heterodimer of the present invention comprises the association of a human BMP-5, as described above, bound through at least one disulfide linkage to a human BMP-7, including, e.g., a monomeric strand of a BMP-7 subunit as described above or an active fragment thereof. In addition, human BMP-5 may be associated with human BMP-8 bound through at least one disulfide linkage to a human BMP-8 subunit or active fragment thereof.

Still another recombinant heterodimer of the present invention comprises the association of a human BMP-6, including, e.g., a monomeric strand of a BMP-6 subunit as described above or an active fragment thereof, bound through at least one disulfide linkage to a human BMP-7, as described above. Another recombinant heterodimer of the present invention comprises the association of a human BMP-6, as described above, bound through one or more covalent, disulfide linkages to a human BMP-8, as described above. Another recombinant heterodimer of the present invention comprises the association of a human BMP-7, as described above bound through one or more covalent, disulfide linkages to a

human BMP-8, as described above.

The disulfide linkages formed between the monomeric strands of the BMPs may occur between one Cys on each strand. Disulfide linkages may form between two Cys on each BMP. Disulfide linkages may form between three Cys on each BMP. Disulfide linkages may form between four Cys on each BMP. Disulfide linkages may form between five Cys on each BMP. Disulfide linkages may form between six Cys on each BMP. Disulfide linkages may form between seven Cys on each BMP. These disulfide linkages may form between adjacent Cys on each BMP or between only selected Cys interspersed within the respective protein sequence. Various heterodimers having the same BMP component strands may form with different numbers of disulfide linkages. Various heterodimers having the same BMP component strands may form with disulfide bonds at different Cys locations. Different heterodimers encompassed by this invention having the same BMP components may differ based upon their recombinant production in mammalian cells, bacterial cells, insect or yeast cells.

These recombinant heterodimers may be characterized by increased alkaline phosphatase activity in the W20 mouse stromal cell line bioassay (Example 8) compared to the individual BMP homodimers, one strand of which forms each heterodimer. Further, these heterodimers are characterized by greater activity in the

W20 bioassay than is provided by simple mixtures of the individual BMP dimers. Preliminary characterization of heterodimers measured on the W20 bioassay have demonstrated that heterodimers of BMP-2 with BMP-5, BMP-6 or BMP-7 are very active. Similarly, heterodimers of BMP-4 with BMP-5, BMP-6 or BMP-7 are strongly active in the W20 bioassay.

Heterodimers of this invention may also be characterized by activity in bone growth and stimulation assays. For example, a heterodimer of this invention is also active in the rat bone formation assay described below in Example 9. The heterodimers are also active in the osteocalcin bioassay described in Example 8. Other characteristics of a heterodimer of this invention include co-precipitation with anti-BMP antibodies to the two different constituent BMPs, as well as characteristic results on Western blots, high pressure liquid chromatography (HPLC) and on two-dimensional gels, with and without reducing conditions.

One embodiment of the method of the present invention for producing recombinant BMP heterodimers involves culturing a suitable cell line, which has been co-transfected with a DNA sequence coding for expression of a first BMP or fragment thereof and a DNA sequence coding for expression of a second BMP or fragment thereof, under the control of known regulatory sequences. The transformed host cells are cultured and the

heterodimeric protein recovered and purified from the culture medium.

In another embodiment of this method which is the presently preferred method of expression of the heterodimers of this invention, a single host cell, e.g., a CHO DUKX cell, is co-transfected with a first DNA molecule containing a DNA sequence encoding one BMP and a second DNA molecule containing a DNA sequence encoding a second selected BMP. One or both plasmids contain a selectable marker that can be used to establish stable cell lines expressing the BMPs. These separate plasmids containing distinct BMP genes on seperate transcription units are mixed and transfected into the CHO cells using conventional protocols. A ratio of plasmids that gives maximal expression of activity in the W20 assay, generally, 1:1, is determined.

For example, as described in detail in Example 3, equal ratios of a plasmid containing the first BMP and a dihydrofolate reductase (DHFR) marker gene and another plasmid containing a second BMP and a DHFR marker gene can be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by calcium phosphate coprecipitation and transfection, electroporation, microinjection, protoplast fusion or lipofection. Individual DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum by conventional means. DHFR+ cells containing increased gene copies can be selected

for propagation in increasing concentrations of methotrexate (MTX) (e.g. sequential steps in 0.02, 0.1, 0.5 and 2.0 uM MTX) according to the procedures of Kaufman and Sharp, J. Mol. Biol., 159:601-629 (1982); and Kaufman et al, Mol. Cell Biol., 5:1750 (1983). Expression of the heterodimer or at least one BMP linked to DHFR should increase with increasing levels of MTX resistance. Cells that stably express either or both BMP/DHFR genes will survive. However at a high frequency, cell lines stably incorporate and express both plasmids that were present during the initial transfection. The conditioned medium is thereafter harvested and the heterodimer isolated by conventional methods and assayed for activity. This approach can be employed with DHFR-deficient cells.

As an alternative embodiment of this method, a DNA molecule containing one selected BMP gene may be transfected into a stable cell line which already expresses another selected BMP gene. For example as described in detail in Example 3 below, a stable CHO cell line expressing BMP-7 with the DHFR marker (designated 7MB9) [Genetics Institute, Inc] is transfected with a plasmid containing BMP-2 and a second selectable marker gene, e.g., neomycin resistance (Neo). After transfection, the cell is cultured and suitable cells selected by treatment with MTX and the antibiotic, G-418. Surviving cells are then screened for the expression of

the heterodimer. This expression system has the advantage of permitting a single step selection.

Alternative dual selection strategies using different cell lines or different markers can also be used. For example, the use of an adenosine deaminase (ADA) marker to amplify the second BMP gene in a stable CHO cell line expressing a different BMP with the DHFR marker may be preferable, since the level of expression can be increased using deoxycoformycin (DCF)-mediated gene amplification. (See the ADA containing plasmid described in Example 1). Alternatively, any BMP cell line made by first using this marker can then be the recipient of a second BMP expression vector containing a distinct marker and selected for dual resistance and BMP coexpression.

Still another embodiment of a method of expressing the heterodimers of this invention includes transfecting the host cell with a single DNA molecule encoding multiple genes for expression either on a single transcription unit or on separate transcription units. Multicistronic expression involves multiple polypeptides encoded within a single transcript, which can be efficiently translated from vectors utilizing a leader sequence, e.g., from the EMC virus, from poliovirus, or from other conventional sourc s of leader sequences. Two BMP genes and a selectable marker can be expressed within a single transcription unit. For example, vectors

containing the configuration BMPx-EMC-BMPy-DHFR or BMPx-EMC-BMPy-EMC-DHFR can be transfected into CHO cells and selected and amplified using the DHFR marker. A plasmid may be constructed which contains DNA sequences encoding two different BMPs, one or more marker genes and a suitable leader or regulatory sequence on a single transcription unit.

Similarly, host cells may be transfected with a single plasmid which contains separate transcription units for each BMP. A selectable marker, e.g., DHFR, can be contained on a another transcription unit, or alternatively as the second cistron on one or both of the BMP genes. These plasmids may be transfected into a selected host cell for expression of the heterodimer, and the heterodimer isolated from the cells or culture medium as described above.

Another embodiment of this expression method involves cell fusion. Two stable cell lines which express selected BMPs, such as a cell line expressing BMP-2 (e.g., 2EG5) and a cell line expressing BMP-7 (e.g., 7MB9), developed using the DHFR/MTX gene amplification system and expressing BMP at high levels, as described in Example 1 and in the above incorporated U.S. applications, can be transfected with one of several dominant marker genes (e.g., neo^r, hygromycin^r, GPT). After sufficient time in coculture (approximately one day) one resultant cell line expressing one BMP and a

dominant marker can be fused with a cell line expressing a different BMP and preferably a different marker using a fusigenic reagent, such as polyethylene glycol, Sendai virus or other known agent.

The resulting cell hybrids expressing both dominant markers and DHFR can be selected using the appropriate culture conditions, and screened for coexpression of the BMPs or their fragments. The selected hybrid cell contains sequences encoding both selected BMPs, and the heterodimer is formed in the cell and then secreted. The heterodimer is obtained from the conditioned medium and isolated and purified therefrom by conventional methods (see e.g., Example 4). The resulting heterodimer may be characterized by methods described herein.

Cell lines generated from the approaches described above can be used to produce co-expressed, heterodimeric BMP polypeptides. The heterodimeric proteins are isolated from the cell medium in a form substantially free from other proteins with which they are co-produced as well as from other contaminants found in the host cells by conventional purification techniques. The presently preferred method of production is co-transfection of different vectors into CHO cells and methotrexate-mediated gene amplification. Stable cell lines may be used to generate conditioned media containing recombinant BMP that can be purified and

assayed for in vitro and in vivo activities. For example, the resulting heterodimer-producing cell lines obtained by any of the methods described herein may be screened for activity by the assays described in Examples 8 and 9, RNA expression, and protein expression by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

The above-described methods of co-expression of the heterodimers of this invention utilize suitable host cells or cell lines. Suitable cell preferably include mammalian cells, such as Chinese hamster ovary cells (CHO). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293:620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U. S. Patent 4,419,446. Other suitable mammalian cell lines are the CV-1 cell line, BHK cell lines and the 293 cell line. The monkey COS-1 cell line is presently believed to be inefficient in BMP heterodimer production.

Many strains of yeast cells known to those skilled in the art may also be available as host cells for expression of the polypeptides of the present invention, e.g., Saccharomyces cerevisiae. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g.,

Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein.

Another method for producing a biologically active heterodimeric protein of this invention may be employed where the host cells are microbial, preferably bacterial cells, in particular E. coli. For example, the various strains of E. coli (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of B. subtilis, Pseudomonas, other bacilli and the like may also be employed in this method.

This method, which may be employed to produce monomers and dimers (both homodimers and heterodimers) is described in European Patent Application No. 433,225, incorporated herein by reference. Briefly, this process involves culturing a microbial host comprising a nucleotide sequence encoding the desired BMP protein linked in the proper reading frame to an expression control sequence which permits expression of the protein and recovering the monomeric, soluble protein. Where the protein is insoluble in the host cells, the water-insoluble protein fraction is isolated from the host cells and the protein is solubilized. After chromatographic purification, the solubilized protein is subjected to selected conditions to obtain the biologically active dimeric configuration of the protein. This process, which may be employed to produce the heterodimers of this invention, is described specifically

in Example 7, for the production of a BMP-2 homodimer.

Another aspect of the present invention provides DNA molecules or plasmid vectors for use in expression of these recombinant heterodimers. These plasmid vectors may be constructed by resort to known methods and available components known to those of skill in the art. In general, to generate a vector useful in the methods of this invention, the DNA encoding the desired BMP protein is transferred into one or more appropriate expression vectors suitable for the selected host cell.

It is presently contemplated that any expression vector suitable for efficient expression in mammalian cells may be employed to produce the recombinant heterodimers of this invention in mammalian host cells. Preferably the vectors contain the selected BMP DNA sequences described above and in the Figures, which encode selected BMP components of the heterodimer. Alternatively, vectors incorporating modified sequences as described in the above-referenced patent applications are also embodiments of the present invention and useful in the production of the vectors.

In addition to the specific vectors described in Example 1, one skilled in the art can construct mammalian expression vectors by employing the sequence of Figures 1-6 or other DNA sequences containing the coding sequences of Figures 1-6 (SEQ ID NOs: 1, 3, 5, 7, 9 and

11), or other modified sequences and known vectors, such as pCD [Okayama et al, Mol. Cell Biol., 2:161-170 (1982)] and pJL3, pJL4 [Gough et al, EMBO J., 4:645-653 (1985)]. The BMP DNA sequences can be modified by removing the non-coding nucleotides on the 5′ and 3′ ends of the coding region. The deleted non-coding nucleotides may or may not be replaced by other sequences known to be beneficial for expression. The transformation of these vectors into appropriate host cells as described above can produce desired heterodimers.

One skilled in the art could manipulate the sequences of Figures 1-6 by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with e.g., yeast or insect regulatory sequences, to create vectors for intracellular or extracellular expression by yeast or insect cells. [See, e.g., procedures described in published European Patent Application 155,476] for expression in insect cells; and procedures described in published PCT application WO86/00639 and European Patent Application EPA 123,289 for expression in yeast cells].

Similarly, bacterial sequences and preference codons may replace sequences in the described and exemplified mammalian vectors to create suitable expression systems for use in the production of BMP monomers in the method described above. For example, the coding sequences could be further manipulated (e.g.,

ligated to other known linkers or modified by deleting non-coding sequences therefrom or altering nucleotides therein by other known techniques). The modified BMP coding sequences could then be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al, Proc. Natl. Acad. Sci. USA, 77:5230-5233 (1980). The exemplary bacterial vector could then be transformed into bacterial host cells and BMP heterodimers expressed thereby. An exemplary vector for microbial, e.g., bacterial, expression is described below in Example 7.

Other vectors useful in the methods of this invention may contain multiple genes in a single transcription unit. For example, a proposed plasmid p7E2D contains the BMP-7 gene followed by the EMC leader sequence, followed by the BMP-2 gene, followed by the DHFR marker gene. Another example is plasmid p7E2ED which contains the BMP-7 gene, the EMC leader, the BMP-2 gene, another EMC leader sequence and the DHFR marker gene. Alternatively, the vector may contain more than one transcription unit. As one example, the plasmid p2ED7ED contains a transcription unit for BMP-2 and a separate transcription unit for BMP-7, i.e., BMP-2-EMC-DHFR and BMP-7-EMC-DHFR. Alternatively, each transcription unit on the plasmid may contain a different marker gene. For example, plasmid p2EN7ED contains BMP-2-EMC-Neo and BMP-7-EMC-DHFR.

Additionally the vectors also contain appropriate expression control sequences which are capable of directing the replication and expression of the BMP in the selected host cells. Useful regulatory sequences for such vectors are known to one of skill in the art and may be selected depending upon the selected host cells. Such selection is routine and does not form part of the present invention. Similarly, the vectors may contain one or more selection markers, such as the antibiotic resistance gene, Neo or selectable markers such as DHFR and ADA. The presently preferred marker gene is DHFR. These marker genes may also be selected by one of skill in the art.

Once they are expressed by one of the methods described above, the heterodimers of this invention may be identified and characterized by application of a variety of assays and procedures. A co-precipitation (immunoprecipitation) assay may be performed with antibodies to each of the BMPs forming the heterodimer. Generally antibodies for this use may be developed by conventional means, e.g., using the selected BMP, fragments thereof, or synthetic BMP peptides as antigen. Antibodies employed in assays are generally polyclonal antibodies made from individual BMP peptides or proteins injected into rabbits according to classical techniqu s. This assay is performed conventionally, and permits the identification of the heterodimer, which is precipitated

by antibodies to both BMP components of the heterodimer. In contrast, only one of the two antibodies causes precipitation of any homodimeric form which may be produced in the process of producing the heterodimer.

Another characterizing assay is a Western assay, employing a precipitating antibody, a probing antibody and a detecting antibody. This assay may also be performed conventionally, by using an antibody to one of the BMPs to precipitate the dimers, which are run on reducing SDS-PAGE for Western analysis. An antibody to the second BMP is used to probe the precipitates on the Western gel for the heterodimer. A detecting antibody, such as a goat-antirabbit antibody labelled with horseradish peroxidase (HRP), is then applied, which will reveal the presence of one of the component subunits of the heterodimer.

Finally, the specific activity of the heterodimer may be quantitated as described in detail in Example 6. Briefly, the amount of each BMP is quantitated using Western blot analysis or pulse labelling and SDS-PAGE analysis in samples of each BMP homodimer and the heterodimer. The W20 activity is also determined as described specifically in Example 8. The relative specific activities may be calculated by the formula: W20 alkaline phosphatase activity/amount of BMP on Western blot or by fluorography. As one example, this formula has been determined for the BMP-2/7 heterodimer,

demonstrating that the heterodimer has an estimated 5 to 50 fold higher specific activity than the BMP-2 homodimer.

The heterodimers of the present invention may have a variety of therapeutic and pharmaceutical uses, e.g., in compositions for wound healing, tissue repair, and in similar compositions which have been indicated for use of the individual BMPs. Increased potency of the heterodimers over the individual BMPs may permit lower dosages of the compositions in which they are contained to be administered to a patient in comparison to dosages of compositions containing only a single BMP. A heterodimeric protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage defects in humans and other animals. Such a preparation employing a heterodimeric protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A heterodimeric protein of this invention may be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an

environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. Heterodimeric polypeptides of the invention may also be useful in the treatment of osteoporosis. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g., European Patent Applications 148,155 and 169,016 for discussions thereof.

The proteins of the invention may also be used in wound healing and related tissue repair. The types of wounds include, but are not limited to burns, incisions and ulcers. (See, e.g., PCT Publication WO84/01106 incorporated by reference herein for discussion of wound healing and related tissue repair).

Additionally, the proteins of the invention may increase neuronal survival and therefore be useful in transplantation and treatment of conditions exhibiting a decrease in neuronal survival.

In view of the usefulness of the heterodimers, therefore, a further aspect of the invention is a therapeutic method and composition for repairing fractures and other conditions related to cartilage and/or bone defects or periodontal diseases. In addition, the invention comprises therapeutic methods and compositions for wound healing and tissue repair. Such compositions comprise a therapeutically effective amount of a heterodimeric protein of the invention in admixture

with a pharmaceutically acceptable vehicle, carrier or matrix. The preparation and formulation of such physiologically acceptable protein compositions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

It is expected that the proteins of the invention may act in concert with other related proteins and growth factors. Therapeutic methods and compositions of the invention therefore comprise a therapeutic amount of a heterodimeric protein of the invention with a therapeutic amount of at least one of the other BMP proteins disclosed in co-owned and concurrently filed U. S. applications described above. Such combinations may comprise separate molecules of the BMP proteins or other heteromolecules of the present invention.

In further compositions, heterodimeric proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-α and TGF-β), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications due to the lack of species specificity in BMP proteins. Particularly domestic animals and thoroughbred horses, in addition to

humans, are desired patients for such treatment with heterodimeric proteins of the present invention.

The therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than the heterodimeric proteins of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the heterodimeric BMP composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the heterodimeric protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical

properties, cosmetic appearance and interface properties. The particular application of the heterodimeric BMP compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applicatons, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the BMP compositions from dissassociating from the matrix.

The dosage regimen of a heterodimeric protein-containing pharmaceutical composition will be determined by the attending physician considering various factors which modify the action of the heterodimeric proteins, e.g. amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the size of a wound, type of damaged tissue, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the BMP proteins in the heterodimer and any additional BMP or other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

The following examples are illustrative of the present invention and do not limit its scope.

EXAMPLE 1 - BMP Vector Constructs and Cell Lines

A. BMP-2 Vectors

The mammalian expression vector pMT2 CXM is a derivative of p91023 (b) [Wong et al, Science, 228:810-815 (1985)] differing from the latter in that it

contains the ampicillin resistance gene (Amp) in place of the tetracycline resistance gene (Tet) and further contains a XhoI site for insertion of cDNA clones. The functional elements of pMT2 CXM have been described [R. J. Kaufman, Proc. Natl. Acad. Sci. USA, 82:689-693 (1985)] and include the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5′ splice site and the majority of the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3′ splice acceptor site, a DHFR insert, the SV40 early polyadenylation site (SV40), and pBR322 sequences needed for propagation in E. coli.

EcoRI digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC 67122, excises the cDNA insert present in pMT2-VWF, yielding pMT2 in linear form. Plasmid pMT2 can be ligated and used to transform E. coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods.

Plasmid pMT2 CXM is then constructed using loopout/in mutagenesis [Morinaga et al, Biotechnology, 84:636 (1984)]. This removes bases 1075 to 1145 relative to the HindIII site near the SV40 origin of replication and enhancer sequences of pMT2. In addition it inserts the following sequence:

5′ PO_4-CATGGGCAGCTCGAG-3′ (SEQ ID NO: 15)

at nucleotide 1145. This sequence contains the recognition site for the restriction endonuclease XhoI.

A derivative of pMT2 CXM, termed plasmid pMT23, contains recognition sites for the restriction endonucleases PstI, EcoRI, SalI and XhoI.

Full length BMP-2 cDNA (Fig. 1) (SEQ ID NO: 1) is released from the λGT10 vector by digestion with EcoRI and subcloned into pSP65 [Promega Biotec, Madison, Wisconsin; see, e.g., Melton et al, Nucl. Acids Res., 12:7035-7056 (1984)] in both orientations yielding pBMP-2 #39-3 or pBMP-2 #39-4.

The majority of the untranslated regions of the BMP-2 cDNA are removed in the following manner. The 5′ sequences are removed between the SalI site in the adapter (present from the original cDNA cloning) and the SalI site 7 base pairs upstream of the initiator ATG by digestion of the pSP65 plasmid containing the BMP-2 cDNA with SalI and religation. The 3′ untranslated region is removed using heteroduplex mutagenesis using the oligonucleotide

5′ GAGGGTTGTGGGTGTCGCTAGTGAGTCGACTACAGCAAAATT 3′.
End SalI
(SEQ ID NO: 16)

The sequence contains the terminal 3′ coding region of the BMP-2 cDNA, followed immediately by a recognition site for SalI. The sequence introduces a SalI site following the termination (TAG) codon.

The SalI fragment of this clone was subcloned into the expression vector pMT23, yielding the vector pMT23-BMP2ΔUT. Restriction enzyme sites flank the BMP-2 coding region in the sequence PstI-EcoRI-SalI-BMP-2 cDNA-SalI-EcoRI-XhoI.

The expression plasmid pED4 [Kaufman et al, Nucl. Acids Res., 19:4485-4490 (1991)] was linearized by digestion with EcoRI and treated with calf intestinal phosphatase. The BMP-2 cDNA gene was excised from pMT23-BMP2ΔUT by digestion with EcoRI and recovery of the 1.2 kb fragment by electrophoresis through a 1.0% low melt agarose gel. The linearized pED4 vector and the EcoRI BMP-2 fragment were ligated together, yielding the BMP-2 expression plasmid pBMP2Δ-EMC.

Another vector pBMP-2Δ-EN contains the same sequences contained within the vector pBMP2Δ-EMC, except the DHFR gene has been replaced by conventional means with the neomycin resistance gene from the Tn5 transposable element.

B. BMP4 Vectors

A BMP-4 cDNA sequence set forth in Figure 2 (SEQ ID NO: 3), in which the 3′ untranslated region is removed, is made via heteroduplex mutagenesis with the mutagenic oligonucleotide:

5′ GGATGTGGGTGCCGCTGACTCTAGAGTCGACGGAATTC 3′
End EcoRI
(SEQ ID NO: 17)

This deletes all of the sequences 3′ to the translation terminator codon of the BMP-4 cDNA, juxtaposing this terminator codon and the vector polylinker sequences. This step is performed in an SP65 vector [Promega Biotech] and may also be conveniently performed in pMT2-derivatives containing the BMP-4 cDNA similar to the BMP2 vectors described above. The 5′ untranslated region is removed using the restriction endonuclease BsmI, which cleaves within the eighth codon of BMP-4 cDNA.

Reconstruction of the first eight codons is accomplished by ligation to oligonucleotides:

EcoRI Initiator BsmI
5′ AATTCACCATGATTCCTGGTAACCGAATGCT 3′ (SEQ ID NO: 18)

and

3′ GTGGTACTAAGGACCATTGGCTTAC 5′ (SEQ ID NO: 19)

These oligonucleotides form a duplex which has a BsmI complementary cohesive end capable of ligation to the BsmI restricted BMP-4 cDNA, and it has an EcoRI complementary cohesive end capable of ligation to the EcoRI restricted vector pMT2. Thus the cDNA for BMP-4 with the 5′ and 3′ untranslated regions deleted, and retaining the entire encoding sequence is contained within an EcoRI restriction fragment of approximately 1.2 kb.

The pMT2 CXM plasmid containing this BMP-4

sequence is designated pXMBMP-4ΔUT. It is digested with EcoRI in order to release the BMP-4 cDNA containing insert from the vector. This insert is subcloned into the EcoRI site of the mammalian expression vector pED4, resulting pBMP4Δ-EMC.

C. BMP-5 Vectors

A BMP-5 cDNA sequence comprising the nucleotide sequence from nucleotide #699 to #2070 of Fig. 5 (SEQ ID NO: 9) is specifically amplified as follows. The oligonucleotides CGACCTGCAGCCACCATGCATCTGACTGTA (SEQ ID NO: 20) and TGCCTGCAGTTTAATATTAGTGGCAGC (SEQ ID NO: 21) are utilized as primers to allow the amplification of nucleotide sequence #699 to #2070 of Fig. 5 from the BMP-5 insert of λ-ZAP clone U2-16 [ATCC #68109]. This procedure introduces the nucleotide sequence CGACCTGCAGCCACC (SEQ ID NO: 22) immediately preceeding nucleotide #699 and the nucleotide sequence CTGCAGGCA immediately following nucleotide #2070. The addition of these sequences results in the creation of PstI restriction endonuclease recognition sites at both ends of the amplified DNA fragment. The resulting amplified DNA product of this procedure is digested with the restriction endonuclease PstI and subcloned into the PstI site of the pMT2 derivative pMT21 [Kaufman, Nucl. Acids Res., 19:4485-4490 (1991)]. The resulting clone is designated H5/5/pMT.

The insert of H5/5/pMT is excised by PstI

digestion and subcloned into the plasmid vector pSP65 [Promega Biotech] at the PstI site, resulting in plasmid BMP5/SP6. BMP5/SP6 and U2-16 are digested with the restriction endonucleases NsiI and NdeI to excise the portion of their inserts corresponding to nucleotides #704 to #1876 of Fig. 5. The resulting 1173 nucleotide NsiI-NdeI fragment of clone U2-16 is ligated into the NsiI-NdeI site of BMP5/SP6 from which the corresponding 1173 nucleotide NsiI-NdeI fragment had been removed. The resulting clone is designated BMP5mix/SP65.

Direct DNA sequence analysis of BMP5mix/SP65 is performed to confirm identity of the nucleotide sequences produced by the amplification to those set forth in Fig. 5. The clone BMP5mix/SP65 is digested with the restriction endonuclease PstI resulting in the excision of an insert comprising the nucleotides #699 to #2070 of Fig. 5 and the additional sequences containing the PstI recognition sites as described above. The resulting 1382 nucleotide PstI fragment is subcloned into the PstI site of the pMT2 derivative pMT21. This clone is designated BMP5mix/pMT21#2.

The same fragment is also subcloned into the PstI site of pED4 to yield the vector designated BMP5mix-EMC-11.

D. <u>BMP-6 Vectors</u>

A BMP-6 cDNA sequence comprising the nucleotide sequence from nucleotide #160 to #1706 of

Fig. 4 (SEQ ID NO: 7) is produced by a series of techniques known to those skilled in the art. The clone BMP6C35 [ATCC 68245] is digested with the restriction endonucleases ApaI and TaqI, resulting in the excision of a 1476 nucleotide portion of the insert comprising nucleotide #231 to #1703 of Fig. 4. Synthetic oligonucleotides with SalI restriction endonuclease site converters are designed to replace those nucleotides corresponding to #160 to #230 and #1704 to #1706 which are not contained in the 1476 ApaI-TaqI fragment of the BMP-6 cDNA sequence.

Oligonucleotide/SalI converters conceived to replace the missing 5′ (TCGACCCACCATGCCGGGGCTGGGGCGGAGGGCGCAGTGGCTGT GCTGGTGGTGGGGGCTGTGCTGCAGCTGCTGCGGGCC (SEQ ID NO: 23) and CGCAGCAGCTGCACAGCAGCCCCCACCACCAGCACAGCCACTGCGCCCTCCGCCCCA GCCCCGGCATGGTGGG) (SEQ ID NO: 24) and 3′ (TCGACTGGTTT (SEQ ID NO: 25) and CGAAACCAG (SEQ ID NO: 26)) sequences are annealed to each other independently. The annealed 5′ and 3′ converters are then ligated to the 1476 nucleotide ApaI-TaqI described above, creating a 1563 nucleotide fragment comprising the nucleotide sequence from #160 to #1706 of Fig. 4 and the additional sequences contrived to create SalI restriction endonuclease sites at both ends. The resulting 1563 nucleotide fragment is subcloned into the SalI site of pSP64 [Promega Biotech, Madison, WI]. This clone is designated BMP6/SP64#15.

DNA sequence analysis of BMP6/SP64#15 is performed to confirm identity of the 5′ and 3′ sequences replaced by the converters to the sequence set forth in Fig. 4. The insert of BMP6/SP64#15 is excised by digestion with the restriction endonuclease SalI. The resulting 1563 nucleotide SalI fragment is subcloned into the XhoI restriction endonuclease site of pMT21 and designated herein as BMP6/pMT21.

The PstI site of pED4 is converted to a SalI site by digestion of the plasmid with PstI and ligation to the converter oligonucleotides:

5′-TCGACAGGCTCGCCTGCA-3′ (SEQ ID NO: 27) and

3′-GTCCGAGCGG-5′ (SEQ ID NO: 28).

The above 1563 nucleotide SalI fragment is also subcloned into the SalI site of this pED4 vector, yielding the expression vector BMP6/EMC.

E. BMP-7 Vectors

A BMP-7 sequence comprising the nucleotide sequence from nucleotide #97 to #1402 of Fig. 3 (SEQ ID NO: 5) is specifically amplified as follows. The oligonucleotides CAGGTCGACCCACCATGCACGTGCGCTCA (SEQ ID NO: 29) and TCTGTCGACCTCGGAGGAGCTAGTGGC (SEQ ID NO: 30) are utilized as primers to allow the amplification of nucleotide sequence #97 to #1402 of Fig. 3 from the insert of clone PEH7-9 [ATCC #68182]. This procedure generates the insertion of the nucleotide sequence CAGGTCGACCCACC immediately preceeding nucleotide #97 and

the insertion of the nucleotide sequence GTCGACAGA immediately following nucleotide #1402. The addition of these sequences results in the creation of a SalI restriction endonuclease recognition site at each end of the amplified DNA fragment. The resulting amplified DNA product of this procedure is digested with the restriction endonuclease SalI and subcloned into the SalI site of the plasmid vector pSP64 [Promega Biotech, Madison, WI] resulting in BMP7/SP6#2.

The clones BMP7/SP6#2 and PEH7-9 are digested with the restriction endonucleases NcoI and StuI to excise the portion of their inserts corresponding to nucleotides #363 to #1081 of Fig. 3. The resulting 719 nucleotide NcoI-StuI fragment of clone PEH7-9 is ligated into the NcoI-StuI site of BMP7/SP6#2 from which the corresponding 719 nucleotide fragment is removed. The resulting clone is designated BMP7mix/SP6.

Direct DNA sequence analysis of BMP7mix/SP6 confirmed identity of the 3′ region to the nucleotide sequence from #1082 to #1402 of Fig. 3, however the 5′ region contained one nucleotide misincorporation.

Amplification of the nucleotide sequence (#97 to #1402 of Fig. 3) utilizing PEH7-9 as a template is repeated as described above. The resulting amplified DNA product of this procedure is digested with the restriction endonucleases SalI and PstI. This dig stion results in the excision of a 747 nucleotide fragment

comprising nucleotide #97 to #833 of Fig. 3 plus the additional sequences of the 5′ priming oligonucleotide used to create the SalI restriction endonuclease recognition site described earlier. This 747 SalI-PstI fragment is subcloned into a SalI-PstI digested pSP65 [Promega Biotech, Madison, WI] vector resulting in 5′BMP7/SP65. DNA sequence analysis demonstrates that the insert of the 5′BMP7/SP65#1 comprises a sequence identical to nucleotide #97 to #362 of Fig. 3.

The clones BMP7mix/SP6 and 5′BMP7/SP65 are digested with the restriction endonucleases SalI and NcoI. The resulting 3′ NcoI-SalI fragment of BMP7mix/SP6 comprising nucleotides #363 to #1402 of Fig. 3 and 5′ SalI-NcoI fragment of 5′BMP7/SP65 comprising nucleotides #97 to #362 of Fig. 3 are ligated together at the NcoI restriction sites to produce a 1317 nucleotide fragment comprising nucleotides #97 to #1402 of Fig. 3 plus the additional sequences derived from the 5′ and 3′ oligonucleotide primers which allows the creation of SalI restriction sites at both ends of this fragment.

This 1317 nucleotide SalI fragment is ligated nto the SalI site of the pMT2 derivative pMT2Cla-2. pMT2Cla-2 is constructed by digesting pMT21 with EcoRV and XhoI, treating the digested DNA with Klenow fragment of DNA polymerase I and ligating ClaI linkers (NEBio Labs, CATCGATG). This removes bases 2171 to 2420 starting from the HindIII site near the SV40 origin of

replication and enhancer sequences of pMT2 and introduces a unique ClaI site, but leaves the adenovirus VAI gene intact, resulting in pMT2Cla-2. This clone is designated BMP-7-pMT2.

The insert of BMP-7-pMT2 is excised by digestion with the restriction endonuclease SalI. The resulting 1317 nucleotide SalI fragment is subcloned into the XhoI restriction endonuclease site of pMT21 to yield the clone BMP-7/pMT21. This SalI fragment is also subcloned into the SalI site of the pED4 vector in which the PstI site was converted into a SalI site as described above, resulting in the vector pBMP7/EMC#4.

F. BMP-8 Vectors

At present no mammalian BMP-8 vectors have been constructed. However, using the sequence of Figure 6 (SEQ ID NO: 11), it is contemplated that vectors similar to those described above for the other BMPs may be readily constructed. A bacterial expression vector similar to the BMP-2 vector described in detail in Example 7 may also be constructed for BMP-8, by introducing a Met before the amino acid #284 Ala of Fig. 6. This sequence of BMP-8 is inserted into the vector pALBP2-781 in place of the BMP-2 sequence. See Example 7.

G. BMP Vectors Containing the Adenosine Deaminase (Ada) Marker

BMP genes were inserted into the vector

pMT3SV2Ada [R. J. Kaufman, Meth. Enz., 185:537-566 (1990)] to yield expression plasmids containing separate transcription units for the BMP cDNA gene and the selectable marker Ada. pMT3SV2Ada contains a polylinker with recognition sites for the enzymes PstI, EcoRI, SalI and XbaI that can be used for insertion of and expression of genes (i.e. BMP) in mammalian cells. In addition, the vector contains a second transcription unit encoding Ada which serves as a dominant and amplifiable marker in mammalian cells.

To construct expression vectors for BMP-5, BMP-6 and BMP-7, individually, the same general method was employed. The gene for BMP 5 (Fig. 5), 6 (Fig. 4) or 7 (Fig. 3) was inserted into the polylinker essentially as described above for the pED4 vector. These vectors can be used for transfection into CHO DUKX cells and subsequent selection and amplification using the Ada marker as previously described [Kaufman et al, Proc. Natl. Acad. Sci. USA, 83:3136-3140 (1986)]. Since each such vector does not contain a DHFR gene, the resultant transformed cells remain DHFR negative and can be subsequently transfected with a second vector containing a different BMP in conjunction with DHFR and amplified with methotrexate.

Alternatively, the pMT3SV2Ada/BMP vectors can be used to transfect stable CHO cell lines previously transfected with a different BMP gene and amplified using

the DHFR/methotrexate system. The resultant transfectants can be subsequently amplified using the Ada system, yielding cell lines that coexpress two different BMP genes, and are amplified using both the DHFR and Ada markers.

H. BMP-Expressing Mammalian Cell Lines

At present, the most desirable mammalian cell lines for use in producing the recombinant homodimers and heterodimers of this invention are the following. These cell lines were prepared by conventional transformation of CHO cells using vectors described above.

The BMP-2 expressing cell line 2EG5 is a CHO cell stably transformed with the vector pBMP2delta-EMC.

The BMP-4 expressing cell line 4E9 is a CHO cell stably transformed with the vector pBMP4delta-EMC.

The BMP-5 expressing cell line 5E10 is a CHO cell stably transformed with the vector BMP5mix-EMC-11 (at a amplification level of 2 micromolar MTX).

The BMP-6 expressing cell line 6HG8 is a CHO cell stably transformed with the vector BMP6/EMC.

The BMP-7 expressing cell line 7MB9 is a CHO cell stably transformed with the vector BMP7/pMT21.

EXAMPLE 2 - TRANSIENT EXPRESSION OF BMP HETERODIMERS

The heterodimers of the present invention may be prepared by co-expression in a transient expression system for screening in the assays of Example 8 by two different techniques as follows.

In the first procedure, the pMT2-derived and EMC-derived expression plasmids described in Example 1 and other similarly derived vectors were constructed which encoded, individually, BMP-2 through BMP-7, and transforming growth factor-beta (TGFβ1). All combinations of pairs of plasmids were mixed in equal proportion and used to co-transfect CHO cells using the DEAE-dextran procedure [Sompayrac and Danna, Proc. Natl. Acad. Sci. USA, 78:7575-7578 (1981); Luthman and Magnusson, Nucl. Acids Res., 11:1295-1308 (1983)]. The cells are grown in alpha Minimal Essential Medium (α-MEM) supplemented with 10% fetal bovine serum, adenosine, deoxyadenosine, thymidine (100 μg/ml each), pen/strep, and glutamine (1 mM).

The addition of compounds such as heparin, suramin and dextran sulfate are desirable in growth medium to increase the amounts of BMP-2 present in the conditioned medium of CHO cells. Similarly responsive to such compounds is BMP-5. Therefore, it is expected that these compounds will be added to growth medium for any heterodimer containing these BMP components. Other BMPs may also be responsive to the effects of these compounds, which are believed to inhibit the interaction of the

mature BMP molecules with the cell surface.

The following day, fresh growth medium, with or without 100 μg/ml heparin, was added. Twenty-four hours later, conditioned medium was harvested.

In some experiments, the conditioned medium was collected minus heparin for the 24-48 hour period post-transfection, and the same plates were then used to generate conditioned medium in the presence of heparin 48-72 hour post-transfection. Controls included transfecting cells with expression plasmids lacking any BMP sequences, transfecting cells with plasmids containing sequences for only a single BMP, or mixing conditioned medium from cells transfected with a single BMP with conditioned medium from cells transfected with a different BMP.

Characterizations of the coexpressed heterodimer BMPs in crude conditioned media, which is otherwise not purified, provided the following results. Transiently coexpressed BMP was assayed for induction of alkaline phosphatase activity on W20 stromal cells, as described in Example 8.

Co-expression of BMP-2 with BMP-5, BMP-6 and BMP-7, and BMP-4 with BMP-5, BMP-6 and BMP-7 yielded more alkaline phosphatase inducing activity in the W20 assay than either of the individual BMP homodimers alone or mixtures of homodimers, as shown below. Maximal activity (in vitro), was obtained when BMP-2 was coexpressed with

BMP-7. Increased activity was also found the heterodimers BMP-2/5; BMP-2/6; BMP-4/5; BMP-4/6; and BMP-4/7.

Condition Medium

	TGF-β	BMP-7	BMP-6	BMP-5	BMP-4	BMP-3	BMP-2
BMP-2	33	240	99	89	53	9	29
BMP-3	–	–	–	–	14	–	
BMP-4	12	115	25	22	24		
BMP-5	–	–	–	–			
BMP-6	–	–	–				
BMP-7	–	–					
TGF-β	-						

Condition Medium + heparin

	TGF-β	BMP-7	BMP-6	BMP-5	BMP-4	BMP-3	BMP-2
BMP-2	88	454	132	127	70	77	169
BMP-3	–	–	–	–	7	–	
BMP-4	7	119	30	41	37		
BMP-5	–	–	–	–			
BMP-6	–	–	–				
BMP-7	–	–					
TGF-β	-						

Units: 1 unit of activity is equivalent to that of 1 ng/ml of rhBMP-2.
–: indicates activity below the detection limit of the assay.

These BMP combinations were subsequently expressed using various ratios of expression plasmids (9:1, 3:1, 1:1, 1:3, 1:9) during the CHO cell transient transfection. The performance of this method using plasmids containing BMP-2 and plasmids containing BMP-7 at plasmid number ratios ranging from 9:1 to 1:9, respectively, demonstrated that the highest activity in

the W20 assay was obtained when approximately the same number of plasmids of each BMP were transfected into the host cell. Ratios of BMP-2 to BMP-7 plasmids of 3:1 to 1:3, respectively, also resulted in increased activity in W20 assay in comparison to host cells transfected with plasmids containing only a single BMP. However, these latter ratios produced less activity than the 1:1 ratio.

Similar ratios may be determined by one of skill in the art for heterodimers consisting of other than BMP-2 and BMP-7. For example, preliminary work on the heterodimer formed between BMP-2 and BMP-6 has indicated that a preferred ratio of plasmids for co-transfection is 3:1, respectively. The determination of preferred ratios for this method is within the skill of the art.

As an alternative means to transiently generate coexpressed BMPs, the stable CHO cell lines identified in Example 1 expressing each BMP-2, BMP-4, BMP-5, BMP-6 and BMP-7, are cocultured for one day, and are then fused with 46.7% polyethylene glycol (PEG). One day post-fusion, fresh medium is added and the heterodimers are harvested 24 hours later for the W20 assay, described in Example 8. The assay results were substantially similar to those described immediately above.

Therefore, all combinations of BMP-2 or 4 coexpressed with either BMP-5, 6 or 7 yielded greater activity than any of the BMP homodimers alone. In

control experiments where each BMP homodimer was expressed alone and conditioned media mixed post harvest, the activity was always intermediate between the individual BMPs, demonstrating that the BMP co-expressed heterodimers yield higher activity than combinations of the individually expressed BMP homodimers.

EXAMPLE 3 - STABLE EXPRESSION OF BMP HETERODIMERS

A. BMP-2/7

Based on the results of the transient assays in Example 2, stable cell lines were made that co-express BMP-2 and BMP-7.

A preferred stable cell line, 2E7E-10, was obtained as follows: Plasmid DNA (a 1:1 mixture of pBMP-7-EMC and pBMP-2-EMC, described in Example 1) is transfected into CHO cells by electroporation [Neuman et al, EMBO J., 1:841-845 (1982)].

Two days later, cells are switched to selective medium containing 10% dialyzed fetal bovine serum and lacking nucleosides. Colonies expressing DHFR are counted 10-14 days later. Individual colonies or pools of colonies are expanded and analyzed for expression of each heterodimer BMP component RNA and protein using standard procedures and are subsequently selected for amplification by growth in increasing concentrations of MTX. Stepwise selection of the preferred clone, termed 2E7E, is carried out up to a concentration of 0.5 μM MTX.

The cell line is then subcloned and assayed for heterodimer 2/7 expression.

Procedures for such assay include Western blot analysis to detect the presence of the component DNA, protein analysis and SDS-PAGE analysis of metabolically labelled protein, W20 assay, and analysis for cartilage and/or bone formation activity using the ectopic rat bone formation assay of Example 9. The presently preferred clonally-derived cell line is identified as 2E7E-10. This cell line secretes BMP-2/7 heterodimer proteins into the media containing 0.5 μM MTX.

The CHO cell line 2E7E-10 is grown in Dulbecco's modified Eagle's medium (DMEM)/Ham's nutrient mixture F-12, 1:1 (vol/vol), supplemented with 10% fetal bovine serum. When the cells are 80 to 100% confluent, the medium is replaced with serum-free DMEM/F-12. Medium is harvested every 24 hours for 4 days. For protein production and purification the cells are cultured serum-free.

While the co-expressing cell line 2E7E-10 preliminarily appears to make lower amounts of BMP protein than the BMP2-expressing cell line 2EG5 described in Example 2, preliminary evidence suggests that the specific activity of the presumptive heterodimer is at least 5-fold greater than BMP-2 homodimer (see Example 6).

To construct another heterodimer producing cell

line, the stable CHO cell line 7MB9, previously transfected with pBMP-7-pMT2, and which expresses BMP-7, is employed. 7MB9 may be amplified and selected to 2 μM methotrexate resistance using the DHFR/MTX system. To generate a stable co-expressing cell line, cell line 7MB9 is transfected with the expression vector pBMP-2Δ-EN (EMC-Neo) containing BMP-2 and the neomycin resistance gene from the Tn5 transposable element. The resulting transfected stable cell line was selected for both G-418 and MTX resistance. Individual clones were picked and analyzed for BMP expression, as described above.

It is anticipated that stable cell lines co-expressing other combinations of BMPs which show enhanced activity by transient coexpression will likewise yield greater activity upon stable expression.

B. BMP-2/6

Based on the results of the transient assays in Example 2, stable cell lines were made that co-express BMP-2 and BMP-6.

A preferred stable cell line, 12C07, was obtained as follows: Plasmid DNA (a 1:3 mixture of pBMP-6-EMC and pBMP-2-EMC, described in Example 1) is transfected into CHO cells by electroporation [Neuman et al, EMBO J., 1:841-845 (1982)].

Two days later, cells are switched to selective medium containing 10% dialyzed fetal bovine serum and lacking nucleosides. Colonies expressing DHFR are

counted 10-14 days later. Individual colonies or pools of colonies are expanded and analyzed for expression of each heterodimer BMP component RNA and protein using standard procedures and are subsequently selected for amplification by growth in increasing concentrations of MTX. Stepwise selection of the preferred clone, termed 12-C, is carried out up to a concentration of 2.0 μM MTX. The cell line is then subcloned and assayed for heterodimer 2/6 expression.

Procedures for such assay include Western blot analysis to detect the presence of the component DNA, protein analysis and SDS-PAGE analysis of metabolically labelled protein, W20 assay, and analysis for cartilage and/or bone formation activity using the ectopic rat bone formation assay of Example 9. The presently preferred clonally-derived cell line is identified as 12C07. This cell line secretes BMP-2/6 heterodimer proteins into the media containing 2.0 μM MTX.

The CHO cell line 12C07 is grown in Dulbecco's modified Eagle's medium (DMEM)/Ham's nutrient mixture F-12, 1:1 (vol/vol), supplemented with 10% fetal bovine serum. When the cells are 80 to 100% confluent, the medium is replaced with serum-free DMEM/F-12. Medium is harvested every 24 hours for 4 days. For protein production and purification the cells are cultured serum-free.

While the co-expressing cell line 12C07

preliminarily appears to make lower amounts of BMP protein than the BMP2-expressing cell line 2EG5 described in Example 2, preliminary evidence suggests that the specific activity of the presumptive heterodimer is at least 3-5-fold greater than BMP-2 homodimer (see Example 6).

To construct another heterodimer producing cell line, the stable CHO cell line 2EG5, previously transfected with pBMP-2-EMC, and which expresses BMP-2, is employed. 2EG5 may be amplified and selected to 2 μM methotrexate resistance using the DHFR/MTX system. To generate a stable co-expressing cell line, cell line 2EG5 is transfected with the expression vector pBMP-6-ada (ada deaminase) containing BMP-6 and the ADA resistance gene. The resulting transfected stable cell line was selected for both DCF and MTX resistance. Individual clones are picked and analyzed for BMP expression, as described above.

It is anticipated that stable cell lines co-expressing other combinations of BMPs which show enhanced activity by transient coexpression will likewise yield greater activity upon stable expression.

EXAMPLE 4-PURIFICATION OF BMP2/7 AND BMP-2/6 HETERODIMER

The same purification procedure is used for BMP-2/6 heterodimer and BMP-2/7 heterodimer. Conditioned media from cultures of cell line 2E7E-10 or 12C07 containing

recombinantly produced BMP heterodimer 2/7V or 2/6, respectively, can be generated from either adherent or suspension cultures. For small to medium scale generation of coexpressed BMP, adherent cultures are seeded into roller bottles and allowed to grow to confluence in alpha-Minimal Eagles Medium [α-MEM, Gibco, Grand Island, NY] containing 10% dialyzed heat-inactivated fetal calf serum [Hazleton, Denver, PA]. The media is then switched to a serum-free, albumin free, low protein medium based on a 50:50 mixture of Delbecco's Modified Eagle's medium and Hams F-12 medium, optionally supplemented with 100 micrograms/ml dextran sulfate. Four or five daily harvests are pooled, and used to purify the recombinant protein.

Conditioned medium from roller bottle cultures obtained as described above was thawed slowly at room temperature and pooled. The pH of the pooled medium was adjusted to pH 8.0 using 1 M Tris, pH 8.0. A column was poured containing Matrex Cellufine Sulfate [Amicon] and equilibrated in 50 mM Tris, pH 8.0.

Upon completion of loading of the medium, the column was washed with buffer containing 50 mM Tris, 0.4 M NaCl, pH 8.0 until the absorbance at 280 nm reached baseline. The column was then washed with 50 mM Tris, pH 8.0 to remove NaCl from the buffer. The resin was then washed with 50 mM Tris, 0.2 M NaCl, 4 M Urea, pH 8.0 until a peak had eluted. The column was then washed into

50 mM Tris, pH 8.0 to remove the urea.

The bound BMP-2/7 or BMP-2/6 was then eluted using 50 mM Tris, 0.5 M NaCl, 0.5 M Arginine, pH 8.0. The eluate was collected as a single pool and may be optionally stored frozen prior to further purification. This Cellufine Sulfate eluate was diluted with 14 volumes of 6M urea and the pH of the sample was then adjusted to 6.0. A hydroxyapatite-Ultrogel [IBF] column was poured and equilibrated with 80 mM potassium phosphate, 6M urea, pH 6.0.

After the completion of sample loading, the column was washed with 10 bed volumes of the equilibration buffer. Bound BMP-2/7 or BMP-2/6 heterodimers were eluted with 5 bed volumes of 100 mM potassium phosphate, 6M urea, pH 7.4. This eluate was loaded directly onto a Vydac C_4 reverse-phase HPLC column equilibrated in water - 0.1% TFA. BMP-2/7 or BMP-2/6 heterodimers were eluted with a gradient of 30-50% acetonitrile in water - 0.1% trifluoroacetic acid.

Fractions containing BMPs are identified by SDS-PAGE in the presence or absence of reductant. The identity of the BMPs with respect to the heterodimers vs. homodimers is determined by 2D-PAGE (+/- reductant). Fractions with heterodimers gave bands which reduce to two spots. Bands from homodimer fractions reduce to a single spot for each BMP species.

The BMP-2/6 heterodimer subunits are analyzed on a protein sequenator. BMP-2/6 heterodimers of the followig species are present: BMP-6 subunit beginning with amino acid #375 Ser-Ala-Ser-Ser in association with BMP-2 subunit beginning with amino acid #283 Gin-Ala-Lys or #249 Ser-Lev-His, though other less abundant species may be present.

It is contemplated that the same or substantially similar purification techniques may be employed for any recombinant BMP heterodimer of this invention. The hydroxyapatite-Ultrogel column may be unnecessary and that the purification scheme may be modified by loading the Cellufine Sulfate eluate directly onto the C_4 reverse-phase HPLC column without use of the former column for BMP2/7 or BMP-2/6 or the other heterodimers of this invention.

EXAMPLE 5 - PROTEIN CHARACTERIZATION

Total protein secreted from the co-expressing cell lines is analyzed after labelling with ^{35}S-methionine or by Western blot analysis using antibodies raised against both BMPs of the heterodimer, e.g., BMP-2 and BMP-7. Together with the alkaline phosphatase assays, the data indicates the presence of the heterodimer and the specific activity. The following specific details are directed towards data collected for the BMP-2/7 and BMP-2/6 heterodimers; however, by application of similar

methods to the other heterodimers described herein, similar results are expected.

A. ^{35}S-Met labelling

Cell lines derived by cotransfection of BMP2A-EMC and BMP7A-EMC expression vectors were pulsed with ^{35}S-methionine for 15 minutes, and chased for 6 hours in serum free media in the presence or absence of heparin. Total secreted protein was analyzed under reducing conditions by PAGE and fluorography. The results demonstrate that several cell lines secrete both BMP-2 and BMP-7 protein. There is a good correlation between the amount of alkaline phosphatase activity and the amount of coexpressed protein.

Several cell lines secrete less total BMP-2 and 7 than the BMP-2-only expressing cell line 2EG5, which produces 10 μg/ml BMP-2. Cell line 2E7E-10 (amplified at a level of 0.5mM MTX) secretes equal proportions of BMP-2 and BMP-7 at about the same overall level of expression as the cell line 2EG5. Cell line 2E7E-10 produces the equivalent of 600 micrograms/ml of BMP-2 homodimer activity in one assay.

Total labelled protein was also analyzed on a two-dimensional non-reducing/reducing gel system to ascertain whether a heterodimer is made. Preliminary results demonstrate the presence of a unique spot in this gel system that is not found in either the BMP-2-only or BMP-7-only cell lines, suggesting the presence of 2/7

heterodimer. The same gel with purified material produced the same results (e.g., two unique spots on the gel) indicative of the presence of the 2/7 heterodimer. The homodimer of BMP2 produced distinct species on this gel system.

In contrast to the recombinant BMP-2/7 purification, BMP-2 homodimers are not detected during the BMP-2/6 preparation; however, significant amounts of BMP-6 homodimers are found. In addition, a significant amount of a -20 amino acid N-terminal truncated form of BMP-6 is found; this could be eliminated by the inclusion of protease inhibitors during cell culture. BMP-2/6 was found to elute two to three fractions later from C4 RP-HPLC than did BMP-2/7.

Amino acid sequencing indicates that the predominant BMP-2/7 heterodimer species comprises a mature BMP-2 subunit [amino acid #283(Gln)-#396(Arg)] and a mature subunit of BMP-7 [#293(Ser)-#431(His)]. BMP-2/6 heterodimer comprises the mature BMP-2 subunit (#283-396) and the mature BMP-6 subunit [#375(Ser)-#513(His)].

B. Immunoprecipitation coupled to Western blot analysis

Conditioned media from a BMP-2-only (2EG5), a BMP-7-only (7MB9), or the 2E7E-10 co-expressing cell line were subjected to immunoprecipitation with either a BMP-2 or BMP-7 antibody (both conventional

polyclonal antibodies raised in rabbits), then analyzed on Western blots probed with either an anti-BMP-2 or anti-BMP-7 antibody. The 2/7 heterodimer precipitates and is reactive on Western blots with both the BMP-2 and BMP-7 antibodies, while either BMP by itself reacts with its specific antibody, but not with the reciprocal antibody.

It has been demonstrated using this strategy that a protein in the co-expressing cell line that is precipitated by the anti-BMP-7 antibody W33 [Genetics Institute, Inc, Cambridge, Massachusetts] and reacts on a Western blot with the anti-BMP-2 antibody W12 or W10 [Genetics Institute, Inc.] is not present in the BMP-2 or 7-only expressing cell lines. This experiment indicates that this protein species is the heterodimeric protein. Conversely, precipitation with W12 and probing with W33 yielded similar results.

EXAMPLE 6 - SPECIFIC ACTIVITY OF HETERODIMERS

A. In vitro Assays

The specific activity of the BMP-2/7 or BMP-2/6 heterodimer and the BMP-2 homodimer secreted into growth medium of the stable cell lines 2E7E-10 and 2EG55, and 12C07 and 2EG5, respectively, were estimated as follows.

The amount of BMP protein in conditioned medium was measured by either Western blot analysis or by

analyzing protein secreted from ^{35}S-methionine labelled cells by PAGE and fluorography. The amount of activity produced by the same cell lines on W20 cells using either the alkaline phosphatase assay or osteocalcin-induction assay was then estimated. The specific activity of the BMP was calculated from the ratio of activity to protein secreted into the growth medium.

In one experiment 2E7E-10 and 2EG5 secreted similar amounts of total BMP proteins as determined by PAGE and fluorography. 2E7E-10 produced about 50-fold more alkaline phosphatase inducing activity the 2EG5, suggesting that the specific activity of the heterodimer is about 50-fold higher than the homodimer.

In another experiment the amount of BMP-2 secreted by 2EG5 was about 50% higher than BMP-2/7 secreted by 2E7E-10, however, 2E7E-10 produced about 10-fold more osteocalcin-inducing activity that 2EG5. From several different experiments of this type the specific activity of the BMP-2/7 heterodimer is estimated to be between 5 to 50 fold higher than the BMP-2 homodimer.

Figures 8 and 9 compare the activity of BMP-2 and BMP-2/7 in the W20 alkaline phosphatase and BGP (Bone Gla Protein, osteocalcin) assays. BMP-2/7 has greatly increased specific activity relative to BMP-2 (Figure 8). From Figure 8, approximately 1.3 ng/ml of BMP-2/7 was sufficient to induce 50% of the maximal alkaline phosphatase response in W-20 cells. A comparable value

for BMP-2 is difficult to calculate, since the alkaline phosphatase response did not maximize, but greater than 30 ng/ml is needed for a half-maximal response. BMP-2/7 thus has a 20 to 30-fold higher specific activity than BMP-2 in the W-20 assay.

As seen in Figure 9, BMP-2/7 was also a more effective stimulator of BGP (bone gla protein, osteocalcin) production than BMP-2 in this experiment. Treating W-20-17 cells with BMP-2/7 for four days resulted in a maximal BGP response with 62 ng/ml, and 11 ng/ml elicits 50% of the maximal BGP response. In contrast, maximal stimulation of BGP synthesis by BMP-2 was not seen with doses up to 468 ng/ml of protein. The minimal dose of BMP-2/7 needed to elicit a BGP response by W-20-17 cells was 3.9 ng/ml, about seven-fold less than the 29 ng/ml required of BMP-2. These results were consistent with the data obtained in the W-20-17 alkaline phosphatase assays for BMP-2 and BMP-2/7.

Preliminary analysis indicates that BMP-2/6 has a specific activity <u>in vitro</u> similar to that of BMP-2/7. The potencies of BMP-2 and BMP-2/6 on induction of alkaline phosphatase production in W-20 is compared, as shown in Figure 12, BMP-2/6 has a higher specific activity than BMP-2 in this assay system. This data is in good agreement with data obtained from the *in vivo* assay of BMP-2 and BMP-2/6).

B. In Vivo Assay

(i) BMP-2/7

The purified BMP-2/7 and BMP-2 were tested in the rat ectopic bone formation assay. A series of different amounts of BMP-2/7 or BMP-2 were implanted in triplicate in rats. After 5 and 10 days, the implants were removed and examined histologically for the presence of bone and cartilage. The histological scores for the amounts of new cartilage and bone formed are summarized in Table A.

Table A

		5 Day Implants		10 Day Implants	
		BMP-2/7	BMP-2	BMP-2/7	BMP-2
0.04	C	± − ±	− − −	± − ±	− − −
	B	− − −	− − −	± − ±	− − −
0.02	C	± 1 ±	− − −	2 1 2	− ± ±
	B	− − −	− − −	1 ± 1	− ± −
1.0	C	1 ± ±	± ± ±	2 2 2	1 1 ±
	B	− − −	− − −	2 3 3	1 1 ±
5.0	C	2 2 1	1 ± 1	1 1 2	1 2 1
	B	± − 1	− − −	4 4 3	2 3 2
25.0	C			± ± 2	2 2 2
	B			4 4 3	3 3 3

The amount of BMP-2/7 required to induce cartilage and bone in the rat ectopic assay is lower than that of BMP-2. Histologically, the appearance of cartilage and bone induced by BMP-2/7 and BMP-2 are identical.

(ii) BMP-2/6

The *in vivo* activity of BMP-2/6 was compared with that of BMP-2 by implantation of various amounts of each BMP for ten days in the rat ectopic bone formation assay. The results of this study (Table B, Figure 13) indicate that BMP-2/6, similar to BMP-2/7, has increased *in vivo* activity relative to BMP-2. The specific activities of BMP-2, BMP-6, and BMP-2/6 are compared in the ectopic bone formation assay ten days after the proteins are implanted. The results of these experiments are shown in Table C and Figure 14. BMP-2/6 is a more potent inducer of bone formation than either BMP-2 or BMP-6. The amount

of bone formation observed with BMP-2/6 was comparable to that observed with equivalent doses of BMP-2/7. The appearance of BMP-2/6 implants is quite similar to implants containing BMP-2 or BMP-2/7.

Table B

Histological scores of implants of BMP 2/6 and BMP-2 in rat ectopic assay (10 day implants).

BMP (μg)	C/B	BMP-2/6	BMP-2
0.04	C	- ± -	- - -
	B	- - -	- - -
0.20	C	1 1 ±	- - -
	B	± ± ±	- - -
1.0	C	1 3 3	1 1 ±
	B	1 2 2	1 1 ±
5.0	C	2 2 2	1 2 2
	B	2 3 3	2 2 2
25.	C	1 1 1	2 2 1
	B	3 3 3	3 3 3

Table C

Histological scores of implants of BMP-2, BMP-6, and BMP-2/6 in rat ectopic assay (10 day implants).

BMP (μg)	C/B	BMP-2	BMP-6	BMP-2/6
0.04	C	- - -	- - -	- - ±
	B	- - -	- - -	- - ±
0.20	C	- - 2	- - -	1 2 2
	B	- - 1	- - -	2 2 2
1.0	C	- ± ±	2 1 1	1 1 1
	B	- ± ±	1 ± ±	3 3 2
5.0	C	2 2 1	3 1 3	± ± 1
	B	1 1 1	2 ± 1	4 5 4
25.	C	± ± ±	± ± ±	± ± ±
	B	5 4 5	4 4 5	4 5 3

EXAMPLE 7 - EXPRESSION OF BMP DIMER IN E. COLI

A biologically active, homodimeric BMP-2 was expressed in E. coli using the techniques described in

European Patent Application 433,255 with minor modifications. Other methods disclosed in the above-referenced European patent application may also be employed to produce heterodimers of the present invention from E. coli. Application of these methods to the heterodimers of this invention is anticipated to produce active BMP heterodimeric proteins from E. coli.

A. BMP-2 Expression Vector

An expression plasmid pALBP2-781 (Figure 7) (SEQ ID NO: 13) was constructed containing the mature portion of the BMP-2 (SEQ ID NO: 14) gene and other sequences which are described in detail below. This plasmid directed the accumulation of 5-10% of the total cell protein as BMP-2 in an E. coli host strain, GI724, described below.

Plasmid pALBP2-781 contains the following principal features. Nucleotides 1-2060 contain DNA sequences originating from the plasmid pUC-18 [Norrander et al, Gene, 26:101-106 (1983)] including sequences containing the gene for β-lactamase which confers resistance to the antibiotic ampicillin in host E. coli strains, and a colE1-derived origin of replication. Nucleotides 2061-2221 contain DNA sequences for the major leftward promoter (pL) of bacteriophage λ [Sanger et al, J. Mol. Biol., 162:729-773 (1982)], including three operator sequences, O_L1, O_L2 and O_L3. The operators are the binding sites for λcI repressor protein,

intracellular levels of which control the amount of transcription initiation from pL. Nucleotides 2222-2723 contain a strong ribosome binding sequence included on a sequence derived from nucleotides 35566 to 35472 and 38137 to 38361 from bacteriophage lambda as described in Sanger et al, J. Mol. Biol., 162:729-773 (1982). Nucleotides 2724-3133 contain a DNA sequence encoding mature BMP-2 protein with an additional 62 nucleotides of 3'-untranslated sequence.

Nucleotides 3134-3149 provide a "Linker" DNA sequence containing restriction endonuclease sites. Nucleotides 3150-3218 provide a transcription termination sequence based on that of the E. coli aspA gene [Takagi et al, Nucl. Acids Res., 13:2063-2074 (1985)]. Nucleotides 3219-3623 are DNA sequences derived from pUC-18.

As described below, when cultured under the appropriate conditions in a suitable E. coli host strain, pALBP2-781 can direct the production of high levels (approximately 10% of the total cellular protein) of BMP-2 protein.

pALBP2-781 was transformed into the E. coli host strain GI724 (F, lacI^q, lacP^{L8}, ampC::λcI$^+$) by the procedure of Dagert and Ehrlich, Gene, 6:23 (1979). [The untransformed host strain E. coli GI724 was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland on January 31, 1991 under ATCC

No. 55151 for patent purposes pursuant to applicable laws and regulations.] Transformants were selected on 1.5% w/v agar plates containing IMC medium, which is composed of M9 medium [Miller, "Experiments in Molecular Genetics", Cold Spring Harbor Laboratory, New York (1972)] supplemented with 0.5% w/v glucose, 0.2% w/v casamino acids and 100 μg/ml ampicillin.

GI724 contains a copy of the wild-type λcI repressor gene stably integrated into the chromosome at the ampC locus, where it has been placed under the transcriptional control of Salmonella typhimurium trp promoter/operator sequences. In GI724, λcI protein is made only during growth in tryptophan-free media, such as minimal media or a minimal medium supplemented with casamino acids such as IMC, described above. Addition of tryptophan to a culture of GI724 will repress the trp promoter and turn off synthesis of λcI, gradually causing the induction of transcription from pL promoters if they are present in the cell.

GI724 transformed with pALBP2-781 was grown at 37°C to an A_{550} of 0.5 (Absorbence at 550 nm) in IMC medium. Tryptophan was added to a final concentration of 100 μg/ml and the culture incubated for a further 4 hours. During this time BMP-2 protein accumulated to approximately 10% of the total cell protein, all in the "inclusion body" fraction.

BMP-2 is recovered in a non-soluble,

monomeric form as follows. Cell disruption and recovery is performed at 4°C. Approximately 9 g of the wet fermented E. coli GI724/pALBP2-781 cells are suspended in 30 mL of 0.1 M Tris/HCl, 10 mM EDTA, 1 mM phenyl methyl sulphonyl fluoride (PMSF), pH 8.3 (disruption buffer). The cells are passed four times through a cell disrupter and the volume is brought to 100 mL with the disruption buffer. The suspension is centrifuged for 20 min. (15,000 x g). The pellet obtained is suspended in 50 mL disruption buffer containing 1 M NaCl and centrifuged for 10 min. as above. The pellet is suspended in 50 mL disruption buffer containing 1% Triton X-100 (Pierce) and again centrifuged for 10 min. as above. The washed pellet is then suspended in 25 mL of 20 mM Tris/HCl, 1 mM EDTA, 1 mM PMSF, 1% DTT, pH 8.3 and homogenized in a glass homogenizer. The resulting suspension contains crude monomeric BMP-2 in a non-soluble form.

Ten mL of the BMP-2 suspension, obtained as described above, are acidified with 10% acetic acid to pH 2.5 and centrifuged in an Eppendorf centrifuge for 10 min. at room temperature. The supernatant is chromatographed. Chromatography was performed on a Sephacryl S-100 HR column (Pharmacia, 2.6 x 83 cm) in 1% acetic acid at a flow rate of 1.4 mL/minute. Fractions containing monomeric, BMP-2 are pooled. This material is used to generate biologically active, homodimer BMP-2.

Biologically active, homodimeric BMP-2 can

be generated from the monomeric BMP-2 obtained following solubilization and purification, described above, as follows.

0.1, 0.5 or 2.5 mg of the BMP-2 is dissolved at a concentration of 20, 100 or 500 μg/mL, respectively, in 50 mM Tris/HCl, pH 8.0, 1 M NaCl, 5 mM EDTA, 2 mM reduced glutathione, 1 mM oxidized glutathione and 33 mM CHAPS [Calbiochem]. After 4 days at 4°C or 23°C, the mixture is diluted 5 to 10 fold with 0.1% TFA.

Purification of biologically active BMP-2 is achieved by subjecting the diluted mixture to reverse phase HPLC on a a Vydac C4 214TP54 column (25 x .46 cm) [The NEST Group, USA] at a flow rate of 1 ml/minute. Buffer A is 0.1% TFA. Buffer B is 90% acetonitrile, and 0.1% TFA. The linear gradient was 0 to 5 minutes at 20% Buffer B; 5 to 10 minutes at 20 to 30 % Buffer B; 10 to 40 minutes at 30 to 60% Buffer B; and 40 to 50 minutes at 60 to 100% Buffer B. Homodimeric BMP-2 is eluted and collected from the HPLC column.

The HPLC fractions are lyophilized to dryness, redissolved in sample buffer (1.5 M Tris-HCl, pH 8.45, 12% glycerol, 4% SDS, .0075% Serva Blue G, .0025% Phenol Red, with or without 100 mM dithiothreitol) and heated for five minutes at 95°C. The running buffer is 100 mM Tris, 100 mM tricine (16% tricine gel) [Novex], 0.1% SDS at pH 8.3. The SDS-PAGE gel is run at 125 volts for 2.5 hours.

The gel is stained for one hour with 200 ml of 0.5% Coomassie Brilliant Blue R-250, 25% isopropanol, 10% acetic acid, heated to 60°C. The gel is then destained with 10% acetic acid, 10% isopropanol until the background is clear.

The reduced material ran at approximately 13kD; the non-reduced material ran at approximately 30 kD, which is indicative of the BMP-2 dimer. This material was later active in the W20 assay of Example 8.

B. BMP-7 Expression Vector

For high level expression of BMP-7 a plasmid pALBMP7-981 was constructed. pAlBMP7-981 is identical to plasmid pALBP2-781 with two exceptions: the BMP-2 gene (residues 2724-3133 of pALBP2-781) is replaced by the mature portion of the BMP-7 gene, deleted for sequenced encoding the first seven residues of the mature BMP-7 protein sequence:

ATGTCTCATAATC GTTCTAAAAC TCCAAAAAAT CAAGAAGCTC TGCGTATGGC

CAACGTGGCA GAGAACAGCA GCAGCGACCA GAGGCAGGCC TGTAAGAAGC

ACGAGCTGTA TGTCAGCTTC CGAGACCTGG GCTGGCAGGA CTGGATCATC

GCGCCTGAAG GCTACGCCGC CTACTACTGT GAGGGGGAGT GTGCCTTCCC

TCTGAACTCC TACATGAACG CCACCAACCA CGCCATCGTG CAGACGCTGG

TCCACTTCAT CAACCCGGAA ACGGTGCCCA AGCCCTGCTG TGCGCCCACG

CAGCTCAATG CCATCTCCGT CCTCTACTTC GATGACAGCT CCAACGTCAT

CCTGAAGAAA TACAGAAACA TGGTGGTCCG GGCCTGTGGC TGCCACTAGC

TCCTCCGAGA ATTCAGACCC TTTGGGGCCA AGTTTTTCTG GATCCT

and the ribosome binding site found between residues 2707 and 2723 in pALBP2-781 is replaced by a different ribosome binding site, based on that found preceding the T7 phage gene 10, of sequence 5′-CAAGAAGGAGATATACAT-3′. The host strain and growth conditions used for the production of BMP-7 were as described for BMP-2.

C. BMP-3 Expression Vector

For high level expression of BMP-3 a plasmid pALB3-782 was constructed. This plasmid is identical to plasmid pALBP2-781, except that the BMP-2 gene (residues 2724-3133 of pALBP2-781) is replaced by a gene encoding a form of mature BMP-3. The sequence of this BMP-3 gene is:

ATGCGTAAAC AATGGATTGA ACCACGTAAC TGTGCTCGTC GTTATCTGAA
AGTAGACTTT GCAGATATTG GCTGGAGTGA ATGGATTATC TCCCCCAAGT
CCTTTGATGC CTATTATTGC TCTGGAGCAT GCCAGTTCCC CATGCCAAAG
TCTTTGAAGC CATCAAATCA TGCTACCATC CAGAGTATAG TGAGAGCTGT
GGGGGTCGTT CCTGGGATTC CTGAGCCTTG CTGTGTACCA GAAAAGATGT
CCTCACTCAG TATTTTATTC TTTGATGAAA ATAAGAATGT AGTGCTTAAA
GTATACCCTA ACATGACAGT AGAGTCTTGC GCTTGCAGAT AACCTGGCAA
AGAACTCATT TGAATGCTTA ATTCAAT

The host strain and growth conditions used for the production of BMP-3 were as described for BMP-2.

D. Expression of a BMP-2/7 Heterodimer in E. coli

Denatured and purified E. coli BMP-2 and BMP-7 monomers were isolated from E. coli inclusion body pellets by acidification and gel filtration as previously as previously described above. 125 ug of each BMP in 1% acetic acid were mixed and taken to dryness in a speed vac. The material was resuspended in 2.5 ml 50 mM Tris, 1.0 NaCl, 5 mM EDTA, 33 mM CHAPS, 2 mM glutathione (reduced), 1 mM glutathione (oxidized), pH 8.0. The sample was incubated at 23 C for one week.

The BMP-2/7 heterodimer was isolated by HPLC on a 25 x 0.46 cm Vydac C4 column. The sample was centrifuged in a microfuge for 5 minutes, and the supernatant was diluted with 22.5 ml 0.1% TFA.

A buffer : 0.1% TFA

B buffer : 0.1% TFA, 95% acetonitrile

1.0 ml/minute

0-5′	20% B
5-10′	20-30% B
10-90′	30-50% B
90-100′	50-100% B

By SDS-PAGE analysis, the BMP-2/7 heterodimer eluted at about 23′.

Figure 10 is a comparison of the W-20 activity of E. coli BMP-2 and BMP-2/7 heterodimer, indicating greater activity of the heterodimer.

F. Expression of BMP-2/3 Heterodimer in E. coli

BMP-2 and BMP-3 monomers were isolated as follows: to 1.0 g of frozen harvested cells expressing either BMP-2 or BMP-3 was added 3.3 ml of 100 mM Tris, 10 mM EDTA, pH 8.3. The cells were resuspended by vortexing vigorously. 33 ul of 100 mM PMSF in isopropanol was added and the cells lysed by one pass through a French pressure cell. The lysate was centrifuged in a microfuge for 20 minutes at 4 C. The supernatant was discarded. The inclusion body pellet was taken up in 8.0 M quanidine hydrochloride, 0.25 M OTT, 0.5 M Tris, 5 mM EDTA, pH 8.5, and heated at 37 C for one hour.

The reduced and denatured BMP monomers were isolated by HPLC on a Supelco C4 guard column as follows:

A buffer : 0.1% TFA

B buffer : 0.1% TFA, 95% acetonitrile

1.0 ml/minute

0-5′ 1% B

5-40′ 1-70% B

40-45′ 70-100% B

Monomeric BMP eluted at 28-30′. Protein concentration was estimated by A280 and the appropriate extinction coefficient.

10 ug of BMP-2 and BMP-3 were combined and taken to dryness in a speed vac. To this was added 50 ul of 50 mM Tris, 1.0 M NaCl, 5 mM EDTA, 33 mM CHAPS, 2 mM reduced glutathione, 1 mM oxidized glutathione, pH 8.5. The sample was incubated at 23 for 3 days. The sample was analyzed by SDS-PAGE on a 16% tricine gel under reducing and nonreducing conditions. The BMP-2/3 heterodimer migrated at about 35 kd nonreduced, and reduced to BMP-2 monomer at about 13 kd and BMP-3 monomer at about 21 kd.

BMP-2/3 heterodimer produced in *E. coli* is tested for *in vivo* activity. (20 μg) at (ten days) is utilized to compare the *in vivo* activity of BMP-2/3 to BMP-2. BMP-2/3 implants showed no cartilage or bone forming activity, while the BMP-2 control implants showed the predicted amounts of bone and cartilage formation. The *in vivo* data obtained with BMP-2/3 is consistent with the *in vitro* data from the W-20 assay.

EXAMPLE 8 - W-20 BIOASSAYS

A. Description of W-20 cells

Use of the W-20 bone marrow stromal cells as an indicator cell line is based upon the conversion of these cells to osteoblast-like cells after treatment with BMP-2 [R. S. Thies et al, "Bone Morphogenetic Protein alters W-20 stromal cell differentiation in vitro", Journal of Bone and Mineral Research, 5(2):305 (1990); and R. S. Thies et al, "Recombinant Human Bone Morphogenetic Protein 2 Induces Osteoblastic Differentiation in W-20-17 Stromal Cells", Endocrinology, in press (1992)]. Specifically, W-20 cells are a clonal bone marrow stromal cell line derived from adult mice by researchers in the laboratory of Dr. D. Nathan, Children's Hospital, Boston, MA. BMP-2 treatment of W-20 cells results in (1) increased alkaline phosphatase production, (2) induction of PTH stimulated cAMP, and (3) induction of osteocalcin synthesis by the cells. While (1) and (2) represent characteristics associated with the osteoblast phenotype, the ability to synthesize osteocalcin is a phenotypic property only displayed by mature osteoblasts. Furthermore, to date we have observed conversion of W-20 stromal cells to osteoblast-like cells only upon treatment with BMPs. In this manner, the in vitro activities displayed by BMP treated W-20 cells correlate with the in vivo bone forming activity known for BMPs.

Below two in vitro assays useful in comparison of BMP activities of novel osteoinductive molecules are described.

B. W-20 Alkaline Phosphatase Assay Protocol

W-20 cells are plated into 96 well tissue culture plates at a density of 10,000 cells per well in 200 µl of media (DME with 10% heat inactivated fetal calf serum, 2 mM glutamine and 100 U/ml + 100 µg/ml streptomycin. The cells are allowed to attach overnight in a 95% air, 5% CO_2 incubator at 37°C.

The 200 µl of media is removed from each well with a multichannel pipettor and replaced with an equal volume of test sample delivered in DME with 10% heat inactivated fetal calf serum, 2 mM glutamine and 1% penicillin-streptomycin. Test substances are assayed in triplicate.

The test samples and standards are allowed a 24 hour incubation period with the W-20 indicator cells. After the 24 hours, plates are removed from the 37°C incubator and the test media are removed from the cells.

The W-20 cell layers are washed 3 times with 200 µl per well of calcium/magnesium free phosphate buffered saline and these washes are discarded.

50 µl of glass distilled water is added to each well and the assay plates are then placed on a dry ice/ethanol bath for quick freezing. Once frozen, the

assay plates are removed from the dry ice/ethanol bath and thawed at 37°C. This step is repeated 2 more times for a total of 3 freeze-thaw procedures. Once complete, the membrane bound alkaline phosphatase is available for measurement.

50 µl of assay mix (50 mM glycine, 0.05% Triton X-100, 4 mM $MgCl_2$, 5 mM p-nitrophenol phosphate, pH = 10.3) is added to each assay well and the assay plates are then incubated for 30 minutes at 37°C in a shaking waterbath at 60 oscillations per minute.

At the end of the 30 minute incubation, the reaction is stopped by adding 100 µl of 0.2 N NaOH to each well and placing the assay plates on ice.

The spectrophotometric absorbance for each well is read at a wavelength of 405 nanometers. These values are then compared to known standards to give an estimate of the alkaline phosphatase activity in each sample. For example, using known amounts of p-nitrophenol phosphate, absorbance values are generated. This is shown in Table I.

Table I

Absorbance Values for Known Standards of P-Nitrophenol Phosphate

P-nitrophenol phosphate umoles	Mean absorbance (405 nm)
0.000	0
0.006	0.261 +/- .024
0.012	0.521 +/- .031
0.018	0.797 +/- .063

0.024	1.074 +/- .061
0.030	1.305 +/- .083

Absorbance values for known amounts of BMP-2 can be determined and converted to μmoles of p-nitrophenol phosphate cleaved per unit time as shown in Table II.

Table II

Alkaline Phosphatase Values for W-20 Cells Treating with BMP-2

BMP-2 concentration ng/ml	Absorbance Reading 405 nmeters	umoles substrate per hour
0	0.645	0.024
1.56	0.696	0.026
3.12	0.765	0.029
6.25	0.923	0.036
12.50	1.121	0.044
25.0	1.457	0.058
50.0	1.662	0.067
100.0	1.977	0.080

These values are then used to compare the activities of known amounts of BMP heterodimers to BMP-2 homodimer.

c. Osteocalcin RIA Protocol

W-20 cells are plated at 10^6 cells per well in 24 well multiwell tissue culture dishes in 2 mls of DME containing 10% heat inactivated fetal calf serum, 2 mM glutamine. The cells are allowed to attach overnight in an atmosphere of 95% air 5% CO_2 at 37°C.

The next day the medium is changed to DME

containing 10% fetal calf serum, 2 mM glutamine and the test substance in a total volume of 2 ml. Each test substance is administered to triplicate wells. The test substances are incubated with the W-20 cells for a total of 96 hours with replacement at 48 hours by the same test medias.

At the end of 96 hours, 50 µl of the test media is removed from each well and assayed for osteocalcin production using a radioimmunoassay for mouse osteocalcin. The details of the assay are described in the kit manufactured by Biomedical Technologies Inc., 378 Page Street, Stoughton, MA 02072. Reagents for the assay are found as product numbers BT-431 (mouse osteocalcin standard), BT-432 (Goat anti-mouse Osteocalcin), BT-431R (iodinated mouse osteocalcin), BT-415 (normal goat serum) and BT-414 (donkey anti goat IgG). The RIA for osteocalcin synthesized by W-20 cells in response to BMP treatment is carried out as described in the protocol provided by the manufacturer.

The values obtained for the test samples are compared to values for known standards of mouse osteocalcin and to the amount of osteocalcin produced by W-20 cells in response to challenge with known amounts of BMP-2. The values for BMP-2 induced osteocalcin synthesis by W-20 cells is shown in Table III.

Table III

Osteocalcin Synthesis by W-20 Cells

BMP-2 Concentration ng/ml	Osteocalcin Synthesis ng/well
0	0.8
2	0.9
4	0.8
8	2.2
16	2.7
31	3.2
62	5.1
125	6.5
250	8.2
500	9.4
1000	10.0

EXAMPLE 9 - ROSEN MODIFIED SAMPATH-REDDI ASSAY

A modified version of the rat bone formation assay described in Sampath and Reddi, Proc. Natl. Acad. Sci. USA, 80:6591-6595 (1983) is used to evaluate bone and/or cartilage activity of BMP proteins. This modified assay is herein called the Rosen-modified Sampath-Reddi assay. The ethanol precipitation step of the Sampath-Reddi procedure is replaced by dialyzing (if the composition is a solution) or diafiltering (if the composition is a suspension) the fraction to be assayed against water. The solution or suspension is then redissolved in 0.1% TFA, and the resulting solution added to 20 mg of rat matrix. A mock rat matrix sample not treated with the protein serves as a control. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are

implanted subcutaneously in the abdominal thoracic area of 21-49 ay old male Long Evans rats. The implants are removed after 7-14 days. Half of each implant is used for alkaline phosphatase analysis [see, A. H. Reddi et al, Proc. Natl. Acad. Sci., 69:1601 (1972)].

The other half of each implant is fixed and processed for histological analysis. 1 μm glycolmethacrylate sections are stained with Von Kossa and acid fuschin to score the amount of induced bone and cartilage formation present in each implant. The terms +1 through +5 represent the area of each histological section of an implant occupied by new bone and/or cartilage cells and matrix. A score of +5 indicates that greater than 50% of the implant is new bone and/or cartilage produced as a direct result of protein in the implant. A score of +4, +3, +2, and +1 would indicate that greater than 40%, 30%, 20% and 10% respectively of the implant contains new cartilage and/or bone.

The heterodimeric BMP proteins of this invention may be assessed for activity on this assay.

Numerous modifications and variations in practice of this invention are expected to occur to those skilled in the art. Such modifications and variations are encompassed within the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Israel, David
Wolfman, Neil M.

(ii) TITLE OF INVENTION: Recombinant Bone Morphogenetic Protein Heterodimers, Compositions and Methods of Use.

(iii) NUMBER OF SEQUENCES: 30

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Legal Affairs, Genetics Institute, Inc.
(B) STREET: 87 CambridgePark Drive
(C) CITY: Cambridge
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02140-2387

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Tape
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Kapinos, Ellen J.
(B) REGISTRATION NUMBER: 32,245
(C) REFERENCE/DOCKET NUMBER: GI-5192B

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617-876-1170
(B) TELEFAX: 617-876-5851

:) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1607 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 356..1543

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG CAGGGAGAAT      60

AACTTGCGCA CCCCACTTTG CGCCGGTGCC TTTGCCCCAG CGGAGCCTGC TTCGCCATCT     120

CCGAGCCCCA CCGCCCCTCC ACTCCTCGGC CTTGCCCGAC ACTGAGACGC TGTTCCCAGC     180

GTGAAAAGAG AGACTGCGCG GCCGGCACCC GGGAGAAGGA GGAGGCAAAG AAAAGGAACG     240

GACATTCGGT CCTTGCGCCA GGTCCTTTGA CCAGAGTTTT TCCATGTGGA CGCTCTTTCA     300

ATGGACGTGT CCCCGCGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT CGACC ATG      358
                                                             Met
                                                              1

GTG GCC GGG ACC CGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC CTC        406
Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val Leu
                5                  10                  15

CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC        454
Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe
        20                  25                  30

GCG GCG GCG TCG TCG GGC CGC CCC TCA TCC CAG CCC TCT GAC GAG GTC        502
Ala Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val
    35                  40                  45

CTG AGC GAG TTC GAG TTG CGG CTG CTC AGC ATG TTC GGC CTG AAA CAG        550
Leu Ser Glu Phe Glu Leu Arg Leu Leu Ser Met Phe Gly Leu Lys Gln
50                  55                  60                  65

AGA CCC ACC CCC AGC AGG GAC GCC GTG GTG CCC CCC TAC ATG CTA GAC        598
Arg Pro Thr Pro Ser Arg Asp Ala Val Val Pro Pro Tyr Met Leu Asp
                70                  75                  80

CTG TAT CGC AGG CAC TCA GGT CAG CCG GGC TCA CCC GCC CCA GAC CAC        646
Leu Tyr Arg Arg His Ser Gly Gln Pro Gly Ser Pro Ala Pro Asp His
            85                  90                  95

CGG TTG GAG AGG GCA GCC AGC CGA GCC AAC ACT GTG CGC AGC TTC CAC        694
Arg Leu Glu Arg Ala Ala Ser Arg Ala Asn Thr Val Arg Ser Phe His
        100                 105                 110

CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG AGT GGG AAA ACA ACC        742
His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr Ser Gly Lys Thr Thr
    115                 120                 125

CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG GAG TTT ATC        790
Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu Glu Phe Ile
130                 135                 140                 145

ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT TTA        838
Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln Met Gln Asp Ala Leu
                150                 155                 160

GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA        886
Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile
            165                 170                 175

AAA CCT GCA ACA GCC AAC TCG AAA TTC CCC GTG ACC AGA CTT TTG GAC        934
Lys Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Arg Leu Leu Asp
```

```
        180                 185                 190

ACC AGG TTG GTG AAT CAG AAT GCA AGC AGG TGG GAA ACT TTT GAT GTC      982
Thr Arg Leu Val Asn Gln Asn Ala Ser Arg Trp Glu Thr Phe Asp Val
    195                 200                 205

ACC CCC GCT GTG ATG CGG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA     1030
Thr Pro Ala Val Met Arg Trp Thr Ala Gln Gly His Ala Asn His Gly
210                 215                 220                 225

TTC GTG GTG GAA GTG GCC CAC TTG GAG GAG AAA CAA GGT GTC TCC AAG     1078
Phe Val Val Glu Val Ala His Leu Glu Glu Lys Gln Gly Val Ser Lys
                230                 235                 240

AGA CAT GTT AGG ATA AGC AGG TCT TTG CAC CAA GAT GAA CAC AGC TGG     1126
Arg His Val Arg Ile Ser Arg Ser Leu His Gln Asp Glu His Ser Trp
            245                 250                 255

TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC CAT GAT GGA AAA GGG     1174
Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly His Asp Gly Lys Gly
        260                 265                 270

CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GCC AAA CAC AAA CAG CGG     1222
His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His Lys Gln Arg
    275                 280                 285

AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC TTC     1270
Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp Phe
290                 295                 300                 305

AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC     1318
Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His
                310                 315                 320

GCC TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG     1366
Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu
            325                 330                 335

AAC TCC ACT AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC     1414
Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn
        340                 345                 350

TCT AAG ATT CCT AAG GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC     1462
Ser Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
    355                 360                 365

TCG ATG CTG TAC CTT GAC GAG AAT GAA AAG GTT GTA TTA AAG AAC TAT     1510
Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys Asn Tyr
370                 375                 380                 385

CAG GAC ATG GTT GTG GAG GGT TGT GGG TGT CGC TAGTACAGCA AAATTAAATA   1563
Gln Asp Met Val Val Glu Gly Cys Gly Cys Arg
                390                 395

CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA AAAA                    1607
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 396 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val
1 5 10 15

Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys
20 25 30

Phe Ala Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu
35 40 45

Val Leu Ser Glu Phe Glu Leu Arg Leu Leu Ser Met Phe Gly Leu Lys
50 55 60

Gln Arg Pro Thr Pro Ser Arg Asp Ala Val Val Pro Pro Tyr Met Leu
65 70 75 80

Asp Leu Tyr Arg Arg His Ser Gly Gln Pro Gly Ser Pro Ala Pro Asp
85 90 95

His Arg Leu Glu Arg Ala Ala Ser Arg Ala Asn Thr Val Arg Ser Phe
100 105 110

His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr Ser Gly Lys Thr
115 120 125

Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu Glu Phe
130 135 140

Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln Met Gln Asp Ala
145 150 155 160

Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile
165 170 175

Ile Lys Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Arg Leu Leu
180 185 190

Asp Thr Arg Leu Val Asn Gln Asn Ala Ser Arg Trp Glu Thr Phe Asp
195 200 205

Val Thr Pro Ala Val Met Arg Trp Thr Ala Gln Gly His Ala Asn His
210 215 220

Gly Phe Val Val Glu Val Ala His Leu Glu Glu Lys Gln Gly Val Ser
225 230 235 240

Lys Arg His Val Arg Ile Ser Arg Ser Leu His Gln Asp Glu His Ser
245 250 255

Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly His Asp Gly Lys
260 265 270

Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His Lys Gln

```
         275                 280                 285

Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp
    290                 295                 300

Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr
305                 310                 315                 320

His Ala Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His
                325                 330                 335

Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val
            340                 345                 350

Asn Ser Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala
        355                 360                 365

Ile Ser Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys Asn
    370                 375                 380

Tyr Gln Asp Met Val Val Glu Gly Cys Gly Cys Arg
385                 390                 395
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1954 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 403..1626

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGCGC GGAGCCCGGC CCGGAAGCTA      60

GGTGAGTGTG GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG     120

AGTATCTAGC TTGTCTCCCC GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC     180

ACAGTCCCCG GCCCTCGCCC AGGTTCACTG CAACCGTTCA GAGGTCCCCA GGAGCTGCTG     240

CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC GTAGTGCCAT CCCGAGCAAC     300

GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG CTGTCAAGAA     360

TCATGGACTG TTATTATATG CCTTGTTTTC TGTCAAGACA CC ATG ATT CCT GGT        414
                                                Met Ile Pro Gly
                                                  1

AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC       462
Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly
  5                  10                  15                  20
```

```
GCG AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC          510
Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala
                25                  30                  35

GAG ATT CAG GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG          558
Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu
            40                  45                  50

CTC CTG CGG GAC TTC GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC          606
Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met Phe Gly Leu Arg
        55                  60                  65

CGC CGC CCG CAG CCT AGC AAG AGT GCC GTC ATT CCG GAC TAC ATG CGG          654
Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro Asp Tyr Met Arg
    70                  75                  80

GAT CTT TAC CGG CTT CAG TCT GGG GAG GAG GAG GAA GAG CAG ATC CAC          702
Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu Glu Gln Ile His
85                  90                  95                  100

AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC AGC CGG GCC AAC ACC          750
Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser Arg Ala Asn Thr
                105                 110                 115

GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC CCA GGG ACC          798
Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile Pro Gly Thr
            120                 125                 130

AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC CCT          846
Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile Pro
        135                 140                 145

GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG          894
Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln
    150                 155                 160

GTG GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT          942
Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile
165                 170                 175                 180

TAT GAG GTT ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC          990
Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile
                185                 190                 195

ACA CGA CTA CTG GAC ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG         1038
Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn Val Thr Arg Trp
            200                 205                 210

GAA ACT TTT GAT GTG AGC CCT GCG GTC CTT CGC TGG ACC CGG GAG AAG         1086
Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp Thr Arg Glu Lys
        215                 220                 225

CAG CCA AAC TAT GGG CTA GCC ATT GAG GTG ACT CAC CTC CAT CAG ACT         1134
Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His Leu His Gln Thr
    230                 235                 240

CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC CGA TCG TTA CCT CAA         1182
Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg Ser Leu Pro Gln
245                 250                 255                 260
```

```
GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC ACC TTT GGC          1230
Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val Thr Phe Gly
                265                 270                 275

CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG CGT          1278
His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys Arg
            280                 285                 290

AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC          1326
Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys
        295                 300                 305

CGG CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC          1374
Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp
    310                 315                 320

TGG ATT GTG GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC          1422
Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp
325                 330                 335                 340

TGC CCC TTT CCA CTG GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT          1470
Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala Ile
                345                 350                 355

GTG CAG ACC CTG GTC AAT TCT GTC AAT TCC AGT ATC CCC AAA GCC TGT          1518
Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala Cys
            360                 365                 370

TGT GTG CCC ACT GAA CTG AGT GCC ATC TCC ATG CTG TAC CTG GAT GAG          1566
Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu
        375                 380                 385

TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG ATG GTA GTA GAG GGA          1614
Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu Gly
    390                 395                 400

TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG ATATACACAC              1666
Cys Gly Cys Arg
405

CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC        1726

ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA        1786

ATCCCTAAAC ATTCACCTTG ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT        1846

TGATCATATA TTTTGACAAA ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG        1906

AGTCATTATT TTAAAAAAAA AAAAAAAACT CTAGAGTCGA CGGAATTC                     1954
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ile Pro Gly Asn Arg Met Leu Met Val Val Leu Leu Cys Gln Val
1 5 10 15

Leu Leu Gly Gly Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys
20 25 30

Lys Lys Val Ala Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly
35 40 45

Gln Ser His Glu Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met
50 55 60

Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro
65 70 75 80

Asp Tyr Met Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu
85 90 95

Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser
100 105 110

Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn
115 120 125

Ile Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu
130 135 140

Ser Ser Ile Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu
145 150 155 160

Phe Arg Glu Gln Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His
165 170 175

Arg Ile Asn Ile Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro
180 185 190

Gly His Leu Ile Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn
195 200 205

Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp
210 215 220

Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His
225 230 235 240

Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg
245 250 255

Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu
260 265 270

Val Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg
275 280 285

Arg Ala Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys
290 295 300

Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val
305 310 315 320

Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr
325 330 335

Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
340 345 350

Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile
355 360 365

Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
370 375 380

Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met
385 390 395 400

Val Val Glu Gly Cys Gly Cys Arg
405

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1448 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 97..1389

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTGACCGAGC GGCGCGGACG GCCGCCTGCC CCCTCTGCCA CCTGGGGCGG TGCGGGCCCG 60

GAGCCCGGAG CCCGGGTAGC GCGTAGAGCC GGCGCG ATG CAC GTG CGC TCA CTG 114
Met His Val Arg Ser Leu
1 5

CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA CCC CTG TTC 162
Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro Leu Phe
10 15 20

CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC GAG GTG CAC 210
Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu Val His
25 30 35

TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG GAG ATG 258
Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg Glu Met
40 45 50

CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC CCG CGC CCG 306
Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro Arg Pro
55 60 65 70

```
CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG CTG GAC CTG          354
His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu Asp Leu
                75                  80                  85

TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC GGC CAG GGC          402
Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly Gly Gln Gly
            90                  95                  100

TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT CTG          450
Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro Leu
        105                 110                 115

GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC ATG GTC ATG          498
Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val Met
    120                 125                 130

AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC CAC CCA CGC          546
Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro Arg
135                 140                 145                 150

TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC CCA GAA GGG          594
Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu Gly
                155                 160                 165

GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC TAC ATC CGG          642
Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile Arg
            170                 175                 180

GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT CAG GTG CTC          690
Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr Gln Val Leu
        185                 190                 195

CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC GAC AGC CGT          738
Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser Arg
    200                 205                 210

ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC ATC ACA GCC          786
Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr Ala
215                 220                 225                 230

ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG GGC CTG CAG          834
Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu Gln
                235                 240                 245

CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG GCG          882
Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu Ala
            250                 255                 260

GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC TTC ATG GTG          930
Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met Val
        265                 270                 275

GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC CGG TCC ACG          978
Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile Arg Ser Thr
    280                 285                 290

GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC AAG AAC CAG         1026
Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn Gln
295                 300                 305                 310
```

```
GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC AGC GAC CAG      1074
Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser Asp Gln
            315                 320                 325

AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC CGA GAC CTG      1122
Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu
        330                 335                 340

GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC GCC TAC TAC      1170
Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr
    345                 350                 355

TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC ACC      1218
Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala Thr
360                 365                 370

AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC CCG GAA ACG      1266
Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Glu Thr
375                 380                 385                 390

GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC ATC TCC GTC      1314
Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val
            395                 400                 405

CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA TAC AGA AAC      1362
Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn
        410                 415                 420

ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC GAGAATTCAG            1409
Met Val Val Arg Ala Cys Gly Cys His
    425                 430

ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTC                           1448
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 431 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
 1               5                  10                  15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
            20                  25                  30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
        35                  40                  45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
    50                  55                  60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
```

65 70 75 80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
85 90 95

Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
100 105 110

Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
115 120 125

Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
130 135 140

Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
145 150 155 160

Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
165 170 175

Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
180 185 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
195 200 205

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
210 215 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225 230 235 240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
245 250 255

Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
260 265 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
275 280 285

Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
290 295 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
305 310 315 320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
325 330 335

Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
340 345 350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
355 360 365

Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
370 375 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln

385 390 395 400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
405 410 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420 425 430

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2923 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: Human placenta

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Stratagene catalog #936203 Human placenta cDNA library
(B) CLONE: BMP6C35

(viii) POSITION IN GENOME:
(C) UNITS: bp

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 160..1701

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1282..1698

(ix) FEATURE:
(A) NAME/KEY: mRNA
(B) LOCATION: 1..2923

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGACCATGAG AGATAAGGAC TGAGGGCCAG GAAGGGGAAG CGAGCCCGCC GAGAGGTGGC 60

GGGGACTGCT CACGCCAAGG GCCACAGCGG CCGCGCTCCG GCCTCGCTCC GCCGCTCCAC 120

GCCTCGCGGG ATCCGCGGGG GCAGCCCGGC CGGGCGGGG ATG CCG GGG CTG GGG 174
Met Pro Gly Leu Gly
-374 -370

CGG AGG GCG CAG TGG CTG TGC TGG TGG TGG GGG CTG CTG TGC AGC TGC 222
Arg Arg Ala Gln Trp Leu Cys Trp Trp Trp Gly Leu Leu Cys Ser Cys
-365 -360 -355

TGC GGG CCC CCG CCG CTG CGG CCG CCC TTG CCC GCT GCC GCG GCC GCC 270

```
Cys Gly Pro Pro Pro Leu Arg Pro Pro Leu Pro Ala Ala Ala Ala Ala
            -350                 -345                 -340

GCC GCC GGG GGG CAG CTG CTG GGG GAC GGC GGG AGC CCC GGC CGC ACG      318
Ala Ala Gly Gly Gln Leu Leu Gly Asp Gly Gly Ser Pro Gly Arg Thr
        -335                 -330                 -325

GAG CAG CCG CCG CCG TCG CCG CAG TCC TCC TCG GGC TTC CTG TAC CGG      366
Glu Gln Pro Pro Pro Ser Pro Gln Ser Ser Ser Gly Phe Leu Tyr Arg
    -320                 -315                 -310

CGG CTC AAG ACG CAG GAG AAG CGG GAG ATG CAG AAG GAG ATC TTG TCG      414
Arg Leu Lys Thr Gln Glu Lys Arg Glu Met Gln Lys Glu Ile Leu Ser
-305                 -300                 -295                 -290

GTG CTG GGG CTC CCG CAC CGG CCC CGG CCC CTG CAC GGC CTC CAA CAG      462
Val Leu Gly Leu Pro His Arg Pro Arg Pro Leu His Gly Leu Gln Gln
                -285                 -280                 -275

CCG CAG CCC CCG GCG CTC CGG CAG CAG GAG GAG CAG CAG CAG CAG CAG      510
Pro Gln Pro Pro Ala Leu Arg Gln Gln Glu Glu Gln Gln Gln Gln Gln
            -270                 -265                 -260

CAG CTG CCT CGC GGA GAG CCC CCT CCC GGG CGA CTG AAG TCC GCG CCC      558
Gln Leu Pro Arg Gly Glu Pro Pro Pro Gly Arg Leu Lys Ser Ala Pro
        -255                 -250                 -245

CTC TTC ATG CTG GAT CTG TAC AAC GCC CTG TCC GCC GAC AAC GAC GAG      606
Leu Phe Met Leu Asp Leu Tyr Asn Ala Leu Ser Ala Asp Asn Asp Glu
    -240                 -235                 -230

GAC GGG GCG TCG GAG GGG GAG AGG CAG CAG TCC TGG CCC CAC GAA GCA      654
Asp Gly Ala Ser Glu Gly Glu Arg Gln Gln Ser Trp Pro His Glu Ala
-225                 -220                 -215                 -210

GCC AGC TCG TCC CAG CGT CGG CAG CCG CCC CCG GGC GCC GCG CAC CCG      702
Ala Ser Ser Ser Gln Arg Arg Gln Pro Pro Pro Gly Ala Ala His Pro
                -205                 -200                 -195

CTC AAC CGC AAG AGC CTT CTG GCC CCC GGA TCT GGC AGC GGC GGC GCG      750
Leu Asn Arg Lys Ser Leu Leu Ala Pro Gly Ser Gly Ser Gly Gly Ala
            -190                 -185                 -180

TCC CCA CTG ACC AGC GCG CAG GAC AGC GCC TTC CTC AAC GAC GCG GAC      798
Ser Pro Leu Thr Ser Ala Gln Asp Ser Ala Phe Leu Asn Asp Ala Asp
        -175                 -170                 -165

ATG GTC ATG AGC TTT GTG AAC CTG GTG GAG TAC GAC AAG GAG TTC TCC      846
Met Val Met Ser Phe Val Asn Leu Val Glu Tyr Asp Lys Glu Phe Ser
    -160                 -155                 -150

CCT CGT CAG CGA CAC CAC AAA GAG TTC AAG TTC AAC TTA TCC CAG ATT      894
Pro Arg Gln Arg His His Lys Glu Phe Lys Phe Asn Leu Ser Gln Ile
-145                 -140                 -135                 -130

CCT GAG GGT GAG GTG GTG ACG GCT GCA GAA TTC CGC ATC TAC AAG GAC      942
Pro Glu Gly Glu Val Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp
                -125                 -120                 -115

TGT GTT ATG GGG AGT TTT AAA AAC CAA ACT TTT CTT ATC AGC ATT TAT      990
```

```
Cys Val Met Gly Ser Phe Lys Asn Gln Thr Phe Leu Ile Ser Ile Tyr
            -110                 -105                 -100

CAA GTC TTA CAG GAG CAT CAG CAC AGA GAC TCT GAC CTG TTT TTG TTG      1038
Gln Val Leu Gln Glu His Gln His Arg Asp Ser Asp Leu Phe Leu Leu
        -95                  -90                  -85

GAC ACC CGT GTA GTA TGG GCC TCA GAA GAA GGC TGG CTG GAA TTT GAC      1086
Asp Thr Arg Val Val Trp Ala Ser Glu Glu Gly Trp Leu Glu Phe Asp
    -80                  -75                  -70

ATC ACG GCC ACT AGC AAT CTG TGG GTT GTG ACT CCA CAG CAT AAC ATG      1134
Ile Thr Ala Thr Ser Asn Leu Trp Val Val Thr Pro Gln His Asn Met
-65                  -60                  -55                  -50

GGG CTT CAG CTG AGC GTG GTG ACA AGG GAT GGA GTC CAC GTC CAC CCC      1182
Gly Leu Gln Leu Ser Val Val Thr Arg Asp Gly Val His Val His Pro
                -45                  -40                  -35

CGA GCC GCA GGC CTG GTG GGC AGA GAC GGC CCT TAC GAT AAG CAG CCC      1230
Arg Ala Ala Gly Leu Val Gly Arg Asp Gly Pro Tyr Asp Lys Gln Pro
            -30                  -25                  -20

TTC ATG GTG GCT TTC TTC AAA GTG AGT GAG GTC CAC GTG CGC ACC ACC      1278
Phe Met Val Ala Phe Phe Lys Val Ser Glu Val His Val Arg Thr Thr
        -15                  -10                  -5

AGG TCA GCC TCC AGC CGG CGC CGA CAA CAG AGT CGT AAT CGC TCT ACC      1326
Arg Ser Ala Ser Ser Arg Arg Arg Gln Gln Ser Arg Asn Arg Ser Thr
    1                   5                   10                  15

CAG TCC CAG GAC GTG GCG CGG GTC TCC AGT GCT TCA GAT TAC AAC AGC      1374
Gln Ser Gln Asp Val Ala Arg Val Ser Ser Ala Ser Asp Tyr Asn Ser
                20                  25                  30

AGT GAA TTG AAA ACA GCC TGC AGG AAG CAT GAG CTG TAT GTG AGT TTC      1422
Ser Glu Leu Lys Thr Ala Cys Arg Lys His Glu Leu Tyr Val Ser Phe
            35                  40                  45

CAA GAC CTG GGA TGG CAG GAC TGG ATC ATT GCA CCC AAG GGC TAT GCT      1470
Gln Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala
        50                  55                  60

GCC AAT TAC TGT GAT GGA GAA TGC TCC TTC CCA CTC AAC GCA CAC ATG      1518
Ala Asn Tyr Cys Asp Gly Glu Cys Ser Phe Pro Leu Asn Ala His Met
    65                  70                  75

AAT GCA ACC AAC CAC GCG ATT GTG CAG ACC TTG GTT CAC CTT ATG AAC      1566
Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Leu Met Asn
80                  85                  90                  95

CCC GAG TAT GTC CCC AAA CCG TGC TGT GCG CCA ACT AAG CTA AAT GCC      1614
Pro Glu Tyr Val Pro Lys Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala
                100                 105                 110

ATC TCG GTT CTT TAC TTT GAT GAC AAC TCC AAT GTC ATT CTG AAA AAA      1662
Ile Ser Val Leu Tyr Phe Asp Asp Asn Ser Asn Val Ile Leu Lys Lys
            115                 120                 125

TAC AGG AAT ATG GTT GTA AGA GCT TGT GGA TGC CAC TAACTCGAAA           1708
```

Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
130 135 140

CCAGATGCTG GGGACACACA TTCTGCCTTG GATTCCTAGA TTACATCTGC CTTAAAAAAA 1768

CACGGAAGCA CAGTTGGAGG TGGGACGATG AGACTTTGAA ACTATCTCAT GCCAGTGCCT 1828

TATTACCCAG GAAGATTTTA AAGGACCTCA TTAATAATTT GCTCACTTGG TAAATGACGT 1888

GAGTAGTTGT TGGTCTGTAG CAAGCTGAGT TTGGATGTCT GTAGCATAAG GTCTGGTAAC 1948

TGCAGAAACA TAACCGTGAA GCTCTTCCTA CCCTCCTCCC CCAAAAACCC ACCAAAATTA 2008

GTTTTAGCTG TAGATCAAGC TATTTGGGGT GTTTGTTAGT AAATAGGGAA AATAATCTCA 2068

AAGGAGTTAA ATGTATTCTT GGCTAAAGGA TCAGCTGGTT CAGTACTGTC TATCAAAGGT 2128

AGATTTTACA GAGAACAGAA ATCGGGGAAG TGGGGGGAAC GCCTCTGTTC AGTTCATTCC 2188

CAGAAGTCCA CAGGACGCAC AGCCCAGGCC ACAGCCAGGG CTCCACGGGG CGCCCTTGTC 2248

TCAGTCATTG CTGTTGTATG TTCGTGCTGG AGTTTTGTTG GTGTGAAAAT ACACTTATTT 2308

CAGCCAAAAC ATACCATTTC TACACCTCAA TCCTCCATTT GCTGTACTCT TTGCTAGTAC 2368

CAAAAGTAGA CTGATTACAC TGAGGTGAGG CTACAAGGGG TGTGTAACCG TGTAACACGT 2428

GAAGGCAGTG CTCACCTCTT CTTTACCAGA ACGGTTCTTT GACCAGCACA TTAACTTCTG 2488

GACTGCCGGC TCTAGTACCT TTTCAGTAAA GTGGTTCTCT GCCTTTTTAC TATACAGCAT 2548

ACCACGCCAC AGGGTTAGAA CCAACGAAGA AAATAAAATG AGGGTGCCCA GCTTATAAGA 2608

ATGGTGTTAG GGGGATGAGC ATGCTGTTTA TGAACGGAAA TCATGATTTC CCTGTAGAAA 2668

GTGAGGCTCA GATTAAATTT TAGAATATTT TCTAAATGTC TTTTTCACAA TCATGTGACT 2728

GGGAAGGCAA TTTCATACTA AACTGATTAA ATAATACATT TATAATCTAC AACTGTTTGC 2788

ACTTACAGCT TTTTTTGTAA ATATAAACTA TAATTTATTG TCTATTTTAT ATCTGTTTTG 2848

CTGTGGCGTT GGGGGGGGGG CCGGGCTTTT GGGGGGGGGG GTTTGTTTGG GGGGTGTCGT 2908

GGTGTGGGCG GGCGG 2923

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 513 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Pro Gly Leu Gly Arg Arg Ala Gln Trp Leu Cys Trp Trp Trp Gly
-374 -370 -365 -360

```
Leu Leu Cys Ser Cys Cys Gly Pro Pro Pro Leu Arg Pro Pro Leu Pro
            -355                 -350                 -345

Ala Ala Ala Ala Ala Ala Ala Gly Gly Gln Leu Leu Gly Asp Gly Gly
        -340                 -335                 -330

Ser Pro Gly Arg Thr Glu Gln Pro Pro Pro Ser Pro Gln Ser Ser Ser
    -325                 -320                 -315

Gly Phe Leu Tyr Arg Arg Leu Lys Thr Gln Glu Lys Arg Glu Met Gln
-310                 -305                 -300                 -295

Lys Glu Ile Leu Ser Val Leu Gly Leu Pro His Arg Pro Arg Pro Leu
                -290                 -285                 -280

His Gly Leu Gln Gln Pro Gln Pro Pro Ala Leu Arg Gln Gln Glu Glu
            -275                 -270                 -265

Gln Gln Gln Gln Gln Gln Leu Pro Arg Gly Glu Pro Pro Pro Gly Arg
        -260                 -255                 -250

Leu Lys Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr Asn Ala Leu Ser
    -245                 -240                 -235

Ala Asp Asn Asp Glu Asp Gly Ala Ser Glu Gly Glu Arg Gln Gln Ser
-230                 -225                 -220                 -215

Trp Pro His Glu Ala Ala Ser Ser Ser Gln Arg Arg Gln Pro Pro Pro
                -210                 -205                 -200

Gly Ala Ala His Pro Leu Asn Arg Lys Ser Leu Leu Ala Pro Gly Ser
            -195                 -190                 -185

Gly Ser Gly Gly Ala Ser Pro Leu Thr Ser Ala Gln Asp Ser Ala Phe
        -180                 -175                 -170

Leu Asn Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu Tyr
    -165                 -160                 -155

Asp Lys Glu Phe Ser Pro Arg Gln Arg His His Lys Glu Phe Lys Phe
-150                 -145                 -140                 -135

Asn Leu Ser Gln Ile Pro Glu Gly Glu Val Val Thr Ala Ala Glu Phe
                -130                 -125                 -120

Arg Ile Tyr Lys Asp Cys Val Met Gly Ser Phe Lys Asn Gln Thr Phe
            -115                 -110                 -105

Leu Ile Ser Ile Tyr Gln Val Leu Gln Glu His Gln His Arg Asp Ser
        -100                 -95                 -90

Asp Leu Phe Leu Leu Asp Thr Arg Val Val Trp Ala Ser Glu Glu Gly
    -85                 -80                 -75

Trp Leu Glu Phe Asp Ile Thr Ala Thr Ser Asn Leu Trp Val Val Thr
-70                 -65                 -60                 -55

Pro Gln His Asn Met Gly Leu Gln Leu Ser Val Val Thr Arg Asp Gly
                -50                 -45                 -40
```

```
Val His Val His Pro Arg Ala Ala Gly Leu Val Gly Arg Asp Gly Pro
          -35                 -30                 -25

Tyr Asp Lys Gln Pro Phe Met Val Ala Phe Phe Lys Val Ser Glu Val
     -20                 -15                 -10

His Val Arg Thr Thr Arg Ser Ala Ser Ser Arg Arg Arg Gln Gln Ser
  -5                  1                   5                  10

Arg Asn Arg Ser Thr Gln Ser Gln Asp Val Ala Arg Val Ser Ser Ala
               15                  20                  25

Ser Asp Tyr Asn Ser Ser Glu Leu Lys Thr Ala Cys Arg Lys His Glu
           30                  35                  40

Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala
      45                  50                  55

Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu Cys Ser Phe Pro
  60                  65                  70

Leu Asn Ala His Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu
75                  80                  85                  90

Val His Leu Met Asn Pro Glu Tyr Val Pro Lys Pro Cys Cys Ala Pro
               95                 100                 105

Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Asn Ser Asn
           110                 115                 120

Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys
      125                 130                 135

His
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2153 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(H) CELL LINE: U2-OS osteosarcoma

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: U2-OS human osteosarcoma cDNA library
(B) CLONE: U2-16

(viii) POSITION IN GENOME:
(C) UNITS: bp

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION: 699..2063

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 1647..2060

(ix) FEATURE:
(A) NAME/KEY: mRNA
(B) LOCATION: 1..2153

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
CTGGTATATT TGTGCCTGCT GGAGGTGGAA TTAACAGTAA GAAGGAGAAA GGGATTGAAT      60

GGACTTACAG GAAGGATTTC AAGTAAATTC AGGGAAACAC ATTTACTTGA ATAGTACAAC     120

CTAGAGTATT ATTTTACACT AAGACGACAC AAAAGATGTT AAAGTTATCA CCAAGCTGCC     180

GGACAGATAT ATATTCCAAC ACCAAGGTGC AGATCAGCAT AGATCTGTGA TTCAGAAATC     240

AGGATTTGTT TTGGAAAGAG CTCAAGGGTT GAGAAGAACT CAAAAGCAAG TGAAGATTAC     300

TTTGGGAACT ACAGTTTATC AGAAGATCAA CTTTTGCTAA TTCAAATACC AAAGGCCTGA     360

TTATCATAAA TTCATATAGG AATGCATAGG TCATCTGATC AAATAATATT AGCCGTCTTC     420

TGCTACATCA ATGCAGCAAA AACTCTTAAC AACTGTGGAT AATTGGAAAT CTGAGTTTCA     480

GCTTTCTTAG AAATAACTAC TCTTGACATA TTCCAAAATA TTTAAAATAG GACAGGAAAA     540

TCGGTGAGGA TGTTGTGCTC AGAAATGTCA CTGTCATGAA AAATAGGTAA ATTTGTTTTT     600

TCAGCTACTG GGAAACTGTA CCTCCTAGAA CCTTAGGTTT TTTTTTTTTT AAGAGGACAA     660

GAAGGACTAA AAATATCAAC TTTTGCTTTT GGACAAAA ATG CAT CTG ACT GTA          713
                                          Met His Leu Thr Val
                                          -316-315

TTT TTA CTT AAG GGT ATT GTG GGT TTC CTC TGG AGC TGC TGG GTT CTA        761
Phe Leu Leu Lys Gly Ile Val Gly Phe Leu Trp Ser Cys Trp Val Leu
    -310                 -305                 -300

GTG GGT TAT GCA AAA GGA GGT TTG GGA GAC AAT CAT GTT CAC TCC AGT        809
Val Gly Tyr Ala Lys Gly Gly Leu Gly Asp Asn His Val His Ser Ser
-295                 -290                 -285                 -280

TTT ATT TAT AGA AGA CTA CGG AAC CAC GAA AGA CGG GAA ATA CAA AGG        857
Phe Ile Tyr Arg Arg Leu Arg Asn His Glu Arg Arg Glu Ile Gln Arg
                -275                 -270                 -265

GAA ATT CTC TCT ATC TTG GGT TTG CCT CAC AGA CCC AGA CCA TTT TCA        905
Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro Arg Pro Phe Ser
            -260                 -255                 -250

CCT GGA AAA ATG ACC AAT CAA GCG TCC TCT GCA CCT CTC TTT ATG CTG        953
Pro Gly Lys Met Thr Asn Gln Ala Ser Ser Ala Pro Leu Phe Met Leu
        -245                 -240                 -235

GAT CTC TAC AAT GCC GAA GAA AAT CCT GAA GAG TCG GAG TAC TCA GTA       1001
```

```
Asp Leu Tyr Asn Ala Glu Glu Asn Pro Glu Glu Ser Glu Tyr Ser Val
    -230                -225                -220

AGG GCA TCC TTG GCA GAA GAG ACC AGA GGG GCA AGA AAG GGA TAC CCA      1049
Arg Ala Ser Leu Ala Glu Glu Thr Arg Gly Ala Arg Lys Gly Tyr Pro
-215                -210                -205                -200

GCC TCT CCC AAT GGG TAT CCT CGT CGC ATA CAG TTA TCT CGG ACG ACT      1097
Ala Ser Pro Asn Gly Tyr Pro Arg Arg Ile Gln Leu Ser Arg Thr Thr
                -195                -190                -185

CCT CTG ACC ACC CAG AGT CCT CCT CTA GCC AGC CTC CAT GAT ACC AAC      1145
Pro Leu Thr Thr Gln Ser Pro Pro Leu Ala Ser Leu His Asp Thr Asn
            -180                -175                -170

TTT CTG AAT GAT GCT GAC ATG GTC ATG AGC TTT GTC AAC TTA GTT GAA      1193
Phe Leu Asn Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu
        -165                -160                -155

AGA GAC AAG GAT TTT TCT CAC CAG CGA AGG CAT TAC AAA GAA TTT CGA      1241
Arg Asp Lys Asp Phe Ser His Gln Arg Arg His Tyr Lys Glu Phe Arg
    -150                -145                -140

TTT GAT CTT ACC CAA ATT CCT CAT GGA GAG GCA GTG ACA GCA GCT GAA      1289
Phe Asp Leu Thr Gln Ile Pro His Gly Glu Ala Val Thr Ala Ala Glu
-135                -130                -125                -120

TTC CGG ATA TAC AAG GAC CGG AGC AAC AAC CGA TTT GAA AAT GAA ACA      1337
Phe Arg Ile Tyr Lys Asp Arg Ser Asn Asn Arg Phe Glu Asn Glu Thr
                -115                -110                -105

ATT AAG ATT AGC ATA TAT CAA ATC ATC AAG GAA TAC ACA AAT AGG GAT      1385
Ile Lys Ile Ser Ile Tyr Gln Ile Ile Lys Glu Tyr Thr Asn Arg Asp
            -100                -95                 -90

GCA GAT CTG TTC TTG TTA GAC ACA AGA AAG GCC CAA GCT TTA GAT GTG      1433
Ala Asp Leu Phe Leu Leu Asp Thr Arg Lys Ala Gln Ala Leu Asp Val
        -85                 -80                 -75

GGT TGG CTT GTC TTT GAT ATC ACT GTG ACC AGC AAT CAT TGG GTG ATT      1481
Gly Trp Leu Val Phe Asp Ile Thr Val Thr Ser Asn His Trp Val Ile
    -70                 -65                 -60

AAT CCC CAG AAT AAT TTG GGC TTA CAG CTC TGT GCA GAA ACA GGG GAT      1529
Asn Pro Gln Asn Asn Leu Gly Leu Gln Leu Cys Ala Glu Thr Gly Asp
-55                 -50                 -45                 -40

GGA CGC AGT ATC AAC GTA AAA TCT GCT GGT CTT GTG GGA AGA CAG GGA      1577
Gly Arg Ser Ile Asn Val Lys Ser Ala Gly Leu Val Gly Arg Gln Gly
                -35                 -30                 -25

CCT CAG TCA AAA CAA CCA TTC ATG GTG GCC TTC TTC AAG GCG AGT GAG      1625
Pro Gln Ser Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Ser Glu
            -20                 -15                 -10

GTA CTT CTT CGA TCC GTG AGA GCA GCC AAC AAA CGA AAA AAT CAA AAC      1673
Val Leu Leu Arg Ser Val Arg Ala Ala Asn Lys Arg Lys Asn Gln Asn
        -5                  1                   5

CGC AAT AAA TCC AGC TCT CAT CAG GAC TCC TCC AGA ATG TCC AGT GTT      1721
```

```
Arg Asn Lys Ser Ser Ser His Gln Asp Ser Ser Arg Met Ser Ser Val
 10                  15                  20                  25

GGA GAT TAT AAC ACA AGT GAG CAA AAA CAA GCC TGT AAG AAG CAC GAA      1769
Gly Asp Tyr Asn Thr Ser Glu Gln Lys Gln Ala Cys Lys Lys His Glu
                 30                  35                  40

CTC TAT GTG AGC TTC CGG GAT CTG GGA TGG CAG GAC TGG ATT ATA GCA      1817
Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala
             45                  50                  55

CCA GAA GGA TAC GCT GCA TTT TAT TGT GAT GGA GAA TGT TCT TTT CCA      1865
Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly Glu Cys Ser Phe Pro
         60                  65                  70

CTT AAC GCC CAT ATG AAT GCC ACC AAC CAC GCT ATA GTT CAG ACT CTG      1913
Leu Asn Ala His Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu
     75                  80                  85

GTT CAT CTG ATG TTT CCT GAC CAC GTA CCA AAG CCT TGT TGT GCT CCA      1961
Val His Leu Met Phe Pro Asp His Val Pro Lys Pro Cys Cys Ala Pro
 90                  95                 100                 105

ACC AAA TTA AAT GCC ATC TCT GTT CTG TAC TTT GAT GAC AGC TCC AAT      2009
Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn
                110                 115                 120

GTC ATT TTG AAA AAA TAT AGA AAT ATG GTA GTA CGC TCA TGT GGC TGC      2057
Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ser Cys Gly Cys
            125                 130                 135

CAC TAATATTAAA TAATATTGAT AATAACAAAA AGATCTGTAT TAAGGTTTAT           2110
His

GGCTGCAATA AAAAGCATAC TTTCAGACAA ACAGAAAAAA AAA                      2153
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 454 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met His Leu Thr Val Phe Leu Leu Lys Gly Ile Val Gly Phe Leu Trp
-316 -315                -310                -305

Ser Cys Trp Val Leu Val Gly Tyr Ala Lys Gly Gly Leu Gly Asp Asn
-300                -295                -290                -285

His Val His Ser Ser Phe Ile Tyr Arg Arg Leu Arg Asn His Glu Arg
                -280                -275                -270

Arg Glu Ile Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
            -265                -260                -255
```

Pro Arg Pro Phe Ser Pro Gly Lys Met Thr Asn Gln Ala Ser Ser Ala
-250 -245 -240

Pro Leu Phe Met Leu Asp Leu Tyr Asn Ala Glu Glu Asn Pro Glu Glu
-235 -230 -225

Ser Glu Tyr Ser Val Arg Ala Ser Leu Ala Glu Glu Thr Arg Gly Ala
-220 -215 -210 -205

Arg Lys Gly Tyr Pro Ala Ser Pro Asn Gly Tyr Pro Arg Arg Ile Gln
-200 -195 -190

Leu Ser Arg Thr Thr Pro Leu Thr Thr Gln Ser Pro Pro Leu Ala Ser
-185 -180 -175

Leu His Asp Thr Asn Phe Leu Asn Asp Ala Asp Met Val Met Ser Phe
-170 -165 -160

Val Asn Leu Val Glu Arg Asp Lys Asp Phe Ser His Gln Arg Arg His
-155 -150 -145

Tyr Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro His Gly Glu Ala
-140 -135 -130 -125

Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Arg Ser Asn Asn Arg
-120 -115 -110

Phe Glu Asn Glu Thr Ile Lys Ile Ser Ile Tyr Gln Ile Ile Lys Glu
-105 -100 -95

Tyr Thr Asn Arg Asp Ala Asp Leu Phe Leu Leu Asp Thr Arg Lys Ala
-90 -85 -80

Gln Ala Leu Asp Val Gly Trp Leu Val Phe Asp Ile Thr Val Thr Ser
-75 -70 -65

Asn His Trp Val Ile Asn Pro Gln Asn Asn Leu Gly Leu Gln Leu Cys
-60 -55 -50 -45

Ala Glu Thr Gly Asp Gly Arg Ser Ile Asn Val Lys Ser Ala Gly Leu
-40 -35 -30

Val Gly Arg Gln Gly Pro Gln Ser Lys Gln Pro Phe Met Val Ala Phe
-25 -20 -15

Phe Lys Ala Ser Glu Val Leu Leu Arg Ser Val Arg Ala Ala Asn Lys
-10 -5 1

Arg Lys Asn Gln Asn Arg Asn Lys Ser Ser Ser His Gln Asp Ser Ser
5 10 15 20

Arg Met Ser Ser Val Gly Asp Tyr Asn Thr Ser Glu Gln Lys Gln Ala
25 30 35

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln
40 45 50

Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly
55 60 65

```
Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
 70                  75                  80

Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys
 85                  90                  95                 100

Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
                105                 110                 115

Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
            120                 125                 130

Arg Ser Cys Gly Cys His
        135
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1003 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: Human Heart

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Human heart cDNA library stratagene catalog #936208
(B) CLONE: hH38

(viii) POSITION IN GENOME:
(C) UNITS: bp

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 8..850

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 427..843

(ix) FEATURE:
(A) NAME/KEY: mRNA
(B) LOCATION: 1..997

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
GAATTCC GAG CCC CAT TGG AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC
        Glu Pro His Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile
        -139            -135                -130
```

```
CCG GCT GGG GAG GCG GTC ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG          9
Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val
-125                 -120                 -115                 -110

CCC AGC ATC CAC CTG CTC AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG         14
Pro Ser Ile His Leu Leu Asn Arg Thr Leu His Val Ser Met Phe Gln
                -105                 -100                 -95

GTG GTC CAG GAG CAG TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT         19
Val Val Gln Glu Gln Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp
            -90                  -85                  -80

CTT CAG ACG CTC CGA GCT GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC         24
Leu Gln Thr Leu Arg Ala Gly Asp Glu Gly Trp Leu Val Leu Asp Val
        -75                  -70                  -65

ACA GCA GCC AGT GAC TGC TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA         28
Thr Ala Ala Ser Asp Cys Trp Leu Leu Lys Arg His Lys Asp Leu Gly
    -60                  -55                  -50

CTC CGC CTC TAT GTG GAG ACT GAG GAT GGG CAC AGC GTG GAT CCT GGC         33
Leu Arg Leu Tyr Val Glu Thr Glu Asp Gly His Ser Val Asp Pro Gly
-45                  -40                  -35                  -30

CTG GCC GGC CTG CTG GGT CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC         38
Leu Ala Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe
                -25                  -20                  -15

GTG GTC ACT TTC TTC AGG GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG         43
Val Val Thr Phe Phe Arg Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg
            -10                  -5                   1

GCA GTG AGG CCA CTG AGG AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG         48
Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu
    5                   10                  15

CCG CAG GCC AAC CGA CTC CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC         52
Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser
20                  25                  30                  35

CAC GGC CGG CAG GTC TGC CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG         57
His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln
                40                  45                  50

GAC CTT GGC TGG CTG GAC TGG GTC ATC GCC CCC CAA GGC TAC TCA GCC         62
Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
            55                  60                  65

TAT TAC TGT GAG GGG GAG TGC TCC TTC CCG CTG GAC TCC TGC ATG AAC         67
Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn
        70                  75                  80

GCC ACC AAC CAC GCC ATC CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA         72
Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
    85                  90                  95

AAC GCA GTC CCC AAG GCG TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC         76
Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
100                 105                 110                 115
```

```
TCT GTG CTC TAC TAT GAC AGC AGC AAC AAC GTC ATC CTG CGC AAG CAC      817
Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
                120                 125                 130

CGC AAC ATG GTG GTC AAG GCC TGC GGC TGC CAC TGAGTCAGCC CGCCCAGCCC    870
Arg Asn Met Val Val Lys Ala Cys Gly Cys His
            135                 140

TACTGCAGCC ACCCTTCTCA TCTGGATCGG GCCCTGCAGA GGCAGAAAAC CCTTAAATGC    930

TGTCACAGCT CAAGCAGGAG TGTCAGGGGC CCTCACTCTC GGTGCCTACT TCCTGTCAGG    990

CTTCTGGGAA TTC                                                      1003
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 281 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Glu Pro His Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala
-139             -135                -130                -125

Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser
            -120                -115                -110

Ile His Leu Leu Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val
        -105                -100                -95

Gln Glu Gln Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln
    -90                 -85                 -80

Thr Leu Arg Ala Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala
-75                 -70                 -65                 -60

Ala Ser Asp Cys Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg
                -55                 -50                 -45

Leu Tyr Val Glu Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala
            -40                 -35                 -30

Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val
        -25                 -20                 -15

Thr Phe Phe Arg Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val
    -10                 -5                  1                   5

Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln
                10                  15                  20

Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly
            25                  30                  35

Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu
```

40 45 50

Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr
55 60 65

Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr
70 75 80 85

Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala
90 95 100

Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val
105 110 115

Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn
120 125 130

Met Val Val Lys Ala Cys Gly Cys His
135 140

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3623 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pALBP2-781

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2724..3071

(ix) FEATURE:
(A) NAME/KEY: terminator
(B) LOCATION: 3150..3218

(ix) FEATURE:
(A) NAME/KEY: RBS
(B) LOCATION: 2222..2723

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT 60

CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT 120

TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT 180

AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT 240

TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG 300

CTGAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA 360

TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC 420

TATGTGGCGC GGTATTATCC CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC 480

ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG 540

GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA 600

ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG 660

GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG 720

ACGAGCGTGA CACCACGATG CCTGTAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACTG 780

GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG 840

TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG 900

GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT 960

CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC 1020

AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT 1080

CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA 1140

TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT 1200

CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTTCTG CGCGTAATCT 1260

GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC 1320

TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC 1380

TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC 1440

TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG 1500

GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT 1560

CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG 1620

AGCATTGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG 1680

GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT 1740

ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTTGTGA TGCTCGTCAG 1800

GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT 1860

GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA 1920

TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT 1980

CAGTGAGCGA GGAAGCGGAA GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC 2040

CGATTCATTA ATGCAGAATT GATCTCTCAC CTACCAAACA ATGCCCCCCT GCAAAAAATA 2100

AATTCATATA AAAAACATAC AGATAACCAT CTGCGGTGAT AAATTATCTC TGGCGGTGTT 2160

```
GACATAAATA CCACTGGCGG TGATACTGAG CACATCAGCA GGACGCACTG ACCACCATGA    2220

AGGTGACGCT CTTAAAAATT AAGCCCTGAA GAAGGGCAGC ATTCAAAGCA GAAGGCTTTG    2280

GGGTGTGTGA TACGAAACGA AGCATTGGCC GTAAGTGCGA TTCCGGATTA GCTGCCAATG    2340

TGCCAATCGC GGGGGGTTTT CGTTCAGGAC TACAACTGCC ACACACCACC AAAGCTAACT    2400

GACAGGAGAA TCCAGATGGA TGCACAAACA CGCCGCCGCG AACGTCGCGC AGAGAAACAG    2460

GCTCAATGGA AAGCAGCAAA TCCCCTGTTG GTTGGGGTAA GCGCAAAACC AGTTCCGAAA    2520

GATTTTTTTA ACTATAAACG CTGATGGAAG CGTTTATGCG GAAGAGGTAA AGCCCTTCCC    2580

GAGTAACAAA AAAACAACAG CATAAATAAC CCCGCTCTTA CACATTCCAG CCCTGAAAAA    2640

GGGCATCAAA TTAAACCACA CCTATGGTGT ATGCATTTAT TTGCATACAT TCAATCAATT    2700

GTTATCTAAG GAAATACTTA CAT ATG CAA GCT AAA CAT AAA CAA CGT AAA         2750
                         Met Gln Ala Lys His Lys Gln Arg Lys
                           1               5

CGT CTG AAA TCT AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC TTC AGT      2798
Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser
 10                  15                  20                  25

GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC      2846
Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala
                 30                  35                  40

TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG AAC      2894
Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn
             45                  50                  55

TCC ACT AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT      2942
Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser
         60                  65                  70

AAG ATT CCT AAG GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG      2990
Lys Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser
     75                  80                  85

ATG CTG TAC CTT GAC GAG AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG      3038
Met Leu Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln
 90                  95                  100                 105

GAC ATG GTT GTG GAG GGT TGT GGG TGT CGC TAGTACAGCA AAATTAAATA        3088
Asp Met Val Val Glu Gly Cys Gly Cys Arg
                 110                 115

CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA AAAATCTAGA GTCGACCTGC    3148

AGTAATCGTA CAGGGTAGTA CAAATAAAAA AGGCACGTCA GATGACGTGC CTTTTTTCTT    3208

GTGAGCAGTA AGCTTGGCAC TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG    3268

CGTTACCCAA CTTAATCGCC TTGCAGCACA TCCCCCTTTC GCCAGCTGGC GTAATAGCGA    3328

AGAGGCCCGC ACCGATCGCC CTTCCCAACA GTTGCGCAGC CTGAATGGCG AATGGCGCCT    3388
```

GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTTCA CACCGCATAT ATGGTGCACT 3448

CTCAGTACAA TCTGCTCTGA TGCCGCATAG TTAAGCCAGC CCCGACACCC GCCAACACCC 3508

GCTGACGCGC CCTGACGGGC TTGTCTGCTC CCGGCATCCG CTTACAGACA AGCTGTGACC 3568

GTCTCCGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGA 3623

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Gln Ala Lys His Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys
1 5 10 15

Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp
20 25 30

Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly Glu Cys
35 40 45

Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val
50 55 60

Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala Cys Cys
65 70 75 80

Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp Glu Asn
85 90 95

Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu Gly Cys
100 105 110

Gly Cys Arg
115

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CATGGGCAGC TGAG 14

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GAGGGTTGTG GGTGTCGCTA GTGAGTCGAC TACAGCAAAT T 41

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGATGTGGGT GCCGCTGACT CTAGAGTCGA CGGAATTC 38

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AATTCACCAT GATTCCTGGT AACCGAATGC T 31

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTGGTACTAA GGACCATTGG CTTAC 25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGACCTGCAG CCATGCATCT GACTGTA 27

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TGCCTGCAGT TTAATATTAG TGGCAGC 27

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGACCTGCAG CCACC 15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCGACCCACC ATGCCGGGGC TGGGGCGGAG GGCGCAGTGG CTGTGCTGGT GGTGGGGGCT 60

GTGCTGCAGC TGCTGCGGGC C 81

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGCAGCAGCT GCACAGCAGC CCCCACCACC AGCACAGCCA CTGCGCCCTC CGCCCCAGCC 60

CCGGCATGGT GGG 73

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCGACTGGTT T 11

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGAAACCAG 9

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCGACAGGCT CGCCTGCA 18

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GTCCGAGCGG 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CAGGTCGACC CACCATGCAC GTGCGCTCA 29

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TCTGTCGACC TCGGAGGAGC TAGTGGC 27

WHAT IS CLAIMED IS:

1. A method for producing a heterodimeric protein having bone stimulating activity comprising culturing a selected host cell containing a sequence encoding a first selected BMP or fragment thereof and a sequence encoding a second selected BMP or fragment thereof, said sequences each being under the control of a suitable regulatory sequence capable of directing co-expression of said proteins, and isolating said heterodimeric protein from the culture medium.

2. The method according to claim 1 wherein said first BMP or fragment thereof is present on a first vector transfected into said host cell and said second BMP or fragment thereof is present on a second vector transfected into said host cell.

3. The method according to claim 1 wherein both said BMPs or fragments thereof are incorporated into a chromosome of said host cell.

4. The method according to claim 1 wherein both BMPs or fragments thereof are present on a single vector.

5. The method according to claim 2 wherein

more than a single copy of the gene encoding each said BMP or fragment thereof is present on each vector.

6. The method according to claim 1 wherein said host cell is a hybrid cell prepared by culturing two fused selected, stable host cells, each host cell transfected with a sequence encoding a selected first or second BMP or fragment thereof, said sequences under the control of a suitable regulatory sequence capable of directing expression of each protein or fragment.

7. The method according to claim 1 wherein said host cell is a mammalian cell.

8. The method according to claim 1 wherein said host cell is an insect cell.

9. The method according to claim 1 wherein said host cell is a yeast cell.

10. A method for producing a heterodimeric protein having bone stimulating activity in a bacterial cell comprising culturing a selected host cell containing a sequence encoding a first selected BMP or fragment thereof under the control of a suitable regulatory sequence capable of directing expression of the protein or protein fragment under conditions suitable for the

formation of a soluble, monomeric protein; culturing a selected host cell containing a sequence encoding a second selected BMP or fragment thereof under the control of a suitable regulatory sequence capable of directing expression of the protein or protein fragment under said conditions to form a second soluble, monomeric protein; and mixing said soluble monomeric proteins under conditions permitting the formation of dimeric proteins associated by at least one covalent disulfide bond; isolating from the mixture a heterodimeric protein.

11. The method according to claim 10 wherein said host cell is E. coli.

12. The method according to claim 10 wherein said conditions comprise treating said protein with a solubilizing agent.

13. A recombinant heterodimeric protein having bone stimulating activity comprising a first protein or fragment of BMP-2 in association with a second protein or fragment thereof selected from the group consisting of BMP-5, BMP-6, BMP-7 and BMP-8.

14. The protein according to claim 13 wherein said second protein is BMP-5.

15. The protein according to claim 13 wherein said second protein is BMP-6.

16. The protein according to claim 13 wherein said second protein is BMP-7.

17. The protein according to claim 13 wherein said second protein is BMP-8.

18. A recombinant heterodimeric protein having bone stimulating activity comprising a protein or fragment of BMP-4 in association with a second protein or fragment thereof selected from the group consisting of BMP-5, BMP-6, BMP-7 and BMP-8.

19. The protein according to claim 18 wherein said second protein is BMP-5.

20. The protein according to claim 18 wherein said second protein is BMP-6.

21. The protein according to claim 18 wherein said second protein is BMP-7.

22. The protein according to claim 18 wherein said second protein is BMP-8.

23. A recombinant heterodimeric protein having bone stimulating activity comprising a protein or fragment of a first BMP in association with a second protein or fragment of a second BMP produced by co-expressing said proteins in a selected host cell.

24. The protein according to claim 23 wherein said first BMP is BMP-2 and said second BMP is BMP-7.

25. A cell line comprising a nucleotide sequence encoding a first BMP or fragment thereof under control of a suitable expression regulatory system and a nucleotide sequence encoding a second BMP or fragment thereof under control of a suitable expression regulatory system, said regulatory systems capable of directing the co-expression of said BMPs or fragments thereof and the formation of heterodimeric protein.

26. The cell line according to claim 25 wherein said nucleotide sequences encoding said first and second BMP proteins are present in a single DNA molecule.

27. The cell line according to claim 25 wherein said nucleotide sequence encoding said first BMP is present on a first DNA molecule and said nucleotide sequence encoding said second BMP is present on a second DNA molecule.

28. The cell line according to claim 26 wherein said single DNA molecule comprises a first transcription unit containing a gene encoding a first BMP or fragment thereof and a second transcription unit containing a gene encoding a second BMP or fragment thereof.

29. The cell line according to claim 26 wherein said single DNA molecule comprises a single transcription unit containing multiple copies of said gene encoding said first BMP or fragments thereof and multiple copies of said gene encoding said second BMP or fragments thereof.

30. A DNA molecule comprising a sequence encoding a first selected BMP or fragment thereof and a sequence encoding a second selected BMP or fragment thereof, said sequences under the control of at least one suitable regulatory sequence capable of directing co-expression of each BMP or fragment thereof.

31. The molecule according to claim 30 comprising a first transcription unit containing a gene encoding a first BMP or fragment thereof and a second transcription unit containing a gene encoding a second BMP or fragment thereof.

32. The molecule according to claim 30 comprising a single transcription unit containing multiple copies of said gene encoding said first BMP or fragments thereof and multiple copies of said gene encoding said second BMP or fragments thereof.

33. The protein according to claim 23 wherein said first BMP is BMP-2 and said second BMP is BMP-6.

34. A recombinant BMP-2 homodimer having bone stimulating activity said homodimer produced in E. coli.

35. A method for producing a homodimeric BMP-2 protein having bone stimulating activity said method comprising culturing E. coli host cells and isolating and purifying said protein from the resulting culture medium.

36. A recombinant heterodimeric protein having bone stimulating activity comprising a first protein or fragment of BMP-2 in association with a second protein or fragment of BMP-2.

FIGURE 1A

```
        10          20          30          40          50          60          70
GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG CAGGGAGAAT AACTTGCGCA

        80          90         100         110         120         130         140
CCCCACTTTG CGCCGGTGCC TTTGCCCCAG CGGAGCCTGC TTCGCCATCT CCGAGCCCCA CCGCCCCTCC

       150         160         170         180         190         200         210
ACTCCTCGGC CTTGCCCGAC ACTGAGACGC TGTTCCCAGC GTGAAAAGAG AGACTGCGCG GCCGGCACCC

       220         230         240         250         260         270         280
GGGAGAAGGA GGAGGCAAAG AAAAGGAACG GACATTCGGT CCTTGCGCCA GGTCCTTTGA CCAGAGTTTT

       290         300         310         320         330         340         350
TCCATGTGGA CGCTCTTTCA ATGGACGTGT CCCCGCGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT

      (1)             370                 385                 400
CGACC ATG GTG GCC GGG ACC CGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC
      MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val

          415                 430                 445
CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC GCG
Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala
                        (24)

460                 475                 490                 505
GCG GCG TCG TCG GGC CGC CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG
Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu

        520                 535                 550                 565
TTC GAG TTG CGG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC ACC CCC AGC
Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser

                580                 595                 610
AGG GAC GCC GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CGC AGG CAC TCA GGT
Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly

    625                 640                 655                 670
CAG CCG GGC TCA CCC GCC CCA GAC CAC CGG TTG GAG AGG GCA GCC AGC CGA GCC
Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala
```

FIGURE 1B

```
                685                   700                   715
AAC ACT GTG CGC AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG
Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr

730                   745                   760                   775
AGT GGG AAA ACA ACC CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG
Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu

          790                   805                   820                   835
GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT
Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala

                850                   865                   880
TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA
Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys

    895                   910                   925                   940
CCT GCA ACA GCC AAC TCG AAA TTC CCC GTG ACC AGA CTT TTG GAC ACC AGG TTG
Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Arg Leu Leu Asp Thr Arg Leu

                955                   970                   985
GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG
Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET

1000                  1015                  1030                  1045
CGG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC GTG GTG GAA GTG GCC CAC
Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His

        1060                  1075                  1090                  1105
TTG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG
Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu
                                                                  (249)

                1120                  1135                  1150
CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC
His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly
                                                       (266)

   1165                  1180                  1195                  1210
CAT GAT GGA AAA GGG CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GCC AAA CAC
His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His
                                                  (283)

            1225                  1240                  1255
AAA CAG CGG AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC
Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp
                                  (296)

1270                  1285                  1300                  1315
TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC
Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala
```

FIGURE 1C

```
        1330                  1345                  1360                  1375
TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG AAC TCC ACT
Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
                1390                  1405                  1420
AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT CCT AAG
Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys

   1435                  1450                  1465                  1480
GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG
Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu

           1495                  1510                  1525
AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG
Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly

1540(396)     1553        1563        1573        1583        1593        1603
TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA
Cys Arg

AAAA
```

FIGURE 2A

```
          10         20         30         40         50         60         70
CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGCGC GGAGCCCGGC CCGGAAGCTA GGTGAGTGTG

          80         90        100        110        120        130        140
GCATCCGAGC TGAGGGACGC GAGCCTGAGA CGCCGCTGCT GCTCCGGCTG AGTATCTAGC TTGTCTCCCC

         150        160        170        180        190        200        210
GATGGGATTC CCGTCCAAGC TATCTCGAGC CTGCAGCGCC ACAGTCCCCG GCCCTCGCCC AGGTTCACTG

         220        230        240        250        260        270        280
CAACCGTTCA GAGGTCCCCA GGAGCTGCTG CTGGCGAGCC CGCTACTGCA GGGACCTATG GAGCCATTCC

         290        300        310        320        330        340        350
GTAGTGCCAT CCCGAGCAAC GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG

         360        370        380        390        400    (1)
CTGTCAAGAA TCATGGACTG TTATTATATG CCTTGTTTTC TGTCAAGACA CC ATG ATT CCT
                                                          MET Ile Pro

     417                   432                   447                   462
GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG
Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala

             477                   492                   507
AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG
Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln

522                   537                   552                   567
GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CGG GAC TTC
Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe

         582                   597                   612                   627
GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CCG CAG CCT AGC AAG
Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys

                  642                   657                   672
AGT GCC GTC ATT CCG GAC TAC ATG CGG GAT CTT TAC CGG CTT CAG TCT GGG GAG
Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu
```

FIGURE 2B

```
    687                 702                 717                 732
GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC
Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala

            747                 762                 777
AGC CGG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC
Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile

792                 807                 822                 837
CCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC
Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile

        852                 867                 882                 897
CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG GTG
Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val

                912                 927                 942
GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT
Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val

    957                 972                 987                1002
ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC ACA CGA CTA CTG GAC
MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp

           1017                1032                1047
ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC CCT
Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro

1062                1077                1092                1107
GCG GTC CTT CGC TGG ACC CGG GAG AAG CAG CCA AAC TAT GGG CTA GCC ATT GAG
Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu

       1122                1137                1152                1167
GTG ACT CAC CTC CAT CAG ACT CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC
Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser

                1182                1197                1212
CGA TCG TTA CCT CAA GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC
Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val

   1227                1242                1257                1272
ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CGG AGG GCC AAG
Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys

           1287                1302                1317
CGT AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG
Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg
   (293)
```

FIGURE 2C

```
1332                1347                1362                1377
CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val

      1392                1407                1422                1437
GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG
Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu

               1452                1467                1482
GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT
Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

   1497                1512                1527                1542
GTC AAT TCC AGT ATC CCC AAA GCC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC
Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile

           1557                1572                1587
TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu

1602                1617          (408)     1636        1646        1656
ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG
MET Val Val Glu Gly Cys Gly Cys Arg

     1666        1676        1686        1696        1706        1716        1726
ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TCCACTCACC CACACACTAC

     1736        1746        1756        1766        1776        1786        1796
ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC

     1806        1816        1826        1836        1846        1856        1866
ATTCACCTTG ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA TTTTGACAAA

     1876        1886        1896        1906        1916        1926        1936
ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAAA AAAAAAAACT

     1946
CTAGAGTCGA CGGAATTC
```

FIGURE 3A

```
         10          20          30          40          50
GTGACCGAGC GGCGCGGACG GCCGCCTGCC CCCTCTGCCA CCTGGGGCGG

         60          70          80          90          99
TGCGGGCCCG GAGCCCGGAG CCCGGGTAGC GCGTAGAGCC GGCGCG ATG
                                                      MET
                                                      (1)

          108         117         126         135         144
CAC GTG CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG
His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala

          153         162         171         180         189
CTC TGG GCA CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe

          198         207         216         225         234
AGC CTG GAC AAC GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC
Ser Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu

          243         252         261         270         279
CGC AGC CAG GAG CGG CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT
Arg Ser Gln Glu Arg Arg Glu MET Gln Arg Glu Ile Leu Ser Ile

          288         297         306         315         324
TTG GGC TTG CCC CAC CGC CCG CGC CCG CAC CTC CAG GGC AAG CAC
Leu Gly Leu Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His

          333         342         351         360         369
AAC TCG GCA CCC ATG TTC ATG CTG GAC CTG TAC AAC GCC ATG GCG
Asn Ser Ala Pro MET Phe MET Leu Asp Leu Tyr Asn Ala MET Ala

          378         387         396         405         414
GTG GAG GAG GGC GGC GGG CCC GGC GGC CAG GGC TTC TCC TAC CCC
Val Glu Glu Gly Gly Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro

          423         432         441         450         459
TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT CTG GCC AGC CTG
Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro Leu Ala Ser Leu

          468         477         486         495         504
CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC ATG GTC ATG AGC TTC
Gln Asp Ser His Phe Leu Thr Asp Ala Asp MET Val MET Ser Phe

          513         522         531         540         549
GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC CAC CCA CGC TAC
Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro Arg Tyr
```

FIGURE 3B

```
         558          567          576          585          594
CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC CCA GAA GGG
His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu Gly

         603          612          621          630          639
GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC TAC ATC
Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile

         648          657          666          675          684
CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT CAG
Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr Gln

         693          702          711          720          729
GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC
Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu

         738          747          756          765          774
GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT
Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe

         783          792          801          810          819
GAC ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC
Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His

         828          837          846          855          864
AAC CTG GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC
Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser

         873          882          891          900          909
ATC AAC CCC AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG
Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln

         918          927          936          945          954
AAC AAG CAG CCC TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC
Asn Lys Gln Pro Phe MET Val Ala Phe Phe Lys Ala Thr Glu Val

         963          972          981          990          999
CAC TTC CGC AGC ATC CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG
His Phe Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln
                        (293)

       1008         1017         1026         1035         1044
AAC CGC TCC AAG ACG CCC AAG AAC CAG GAA GCC CTG CGG ATG GCC
Asn Arg Ser Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg MET Ala

       1053         1062         1071         1080         1089
AAC GTG GCA GAG AAC AGC AGC AGC GAC CAG AGG CAG GCC TGT AAG
Asn Val Ala Glu Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys
```

FIGURE 3C

```
      1098        1107        1116        1125        1134
AAG CAC GAG CTG TAT GTC AGC TTC CGA GAC CTG GGC TGG CAG GAC
Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln Asp

      1143        1152        1161        1170        1179
TGG ATC ATC GCG CCT GAA GGC TAC GCC GCC TAC TAC TGT GAG GGG
Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly

      1188        1197        1206        1215        1224
GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC ACC AAC CAC
Glu Cys Ala Phe Pro Leu Asn Ser Tyr MET Asn Ala Thr Asn His

      1233        1242        1251        1260        1269
GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC CCG GAA ACG GTG
Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Ile Ser Val

      1278        1287        1296        1305        1314
CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC ATC TCC GTC
Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val

      1323        1332        1341        1350        1359
CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA TAC AGA
Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg

      1368        1377        1386        1399
AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC
Asn MET Val Val Arg Ala Cys Gly Cys His
                                        (431)

      1409        1419        1429        1439        1448
GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGGTC
```

FIGURE 4A

```
          10          20          30          40          50
CGACCATGAG AGATAAGGAC TGAGGGCCAG GAAGGGGAAG CGAGCCCGCC

          60                  70          80          90          100
GAGAGGTGGC GGGGACTGCT CACGCCAAGG GCCACAGCGG CCGCGCTCCG

         110         120         130         140         150
GCCTCGCTCC GCCGCTCCAC GCCTCGCGGG ATCCGCGGGG GCAGCCCGGC

       159         168         177         186         195
CGGGCGGGG ATG CCG GGG CTG GGG CGG AGG GCG CAG TGG CTG TGC
          MET Pro Gly Leu Gly Arg Arg Ala Gln Trp Leu Cys
          (1)

         204         213         222         231         240
TGG TGG TGG GGG CTG CTG TGC AGC TGC TGC GGG CCC CCG CCG CTG
Trp Trp Trp Gly Leu Leu Cys Ser Cys Cys Gly Pro Pro Pro Leu

         249         258         267         276         285
CGG CCG CCC TTG CCC GCT GCC GCG GCC GCC GCC GCC GGG GGG CAG
Arg Pro Pro Leu Pro Ala Ala Ala Ala Ala Ala Ala Gly Gly Gln

         294         303         312         321         330
CTG CTG GGG GAC GGC GGG AGC CCC GGC CGC ACG GAG CAG CCG CCG
Leu Leu Gly Asp Gly Gly Ser Pro Gly Arg Thr Glu Gln Pro Pro

         339         348         357         366         375
CCG TCG CCG CAG TCC TCC TCG GGC TTC CTG TAC CGG CGG CTC AAG
Pro Ser Pro Gln Ser Ser Ser Gly Phe Leu Tyr Arg Arg Leu Lys

         384         393         402         411         420
ACG CAG GAG AAG CGG GAG ATG CAG AAG GAG ATC TTG TCG GTG CTG
Thr Gln Glu Lys Arg Glu MET Gln Lys Glu Ile Leu Ser Val Leu

         429         438         447         456         465
GGG CTC CCG CAC CGG CCC CGG CCC CTG CAC GGC CTC CAA CAG CCG
Gly Leu Pro His Arg Pro Arg Pro Leu His Gly Leu Gln Gln Pro
```

FIGURE 4B

```
          474         483         492         501         510
CAG CCC CCG GCG CTC CGG CAG CAG GAG GAG CAG CAG CAG CAG CAG
Gln Pro Pro Ala Leu Arg Gln Gln Glu Glu Gln Gln Gln Gln Gln

          519         528         537         546         555
CAG CTG CCT CGC GGA GAG CCC CCT CCC GGG CGA CTG AAG TCC GCG
Gln Leu Pro Arg Gly Glu Pro Pro Pro Gly Arg Leu Lys Ser Ala

          564         573         582         591         600
CCC CTC TTC ATG CTG GAT CTG TAC AAC GCC CTG TCC GCC GAC AAC
Pro Leu Phe MET Leu Asp Leu Tyr Asn Ala Leu Ser Ala Asp Asn

          609         618         627         636         645
GAC GAG GAC GGG GCG TCG GAG GGG GAG AGG CAG CAG TCC TGG CCC
Asp Glu Asp Gly Ala Ser Glu Gly Glu Arg Gln Gln Ser Trp Pro

          654         663         672         681         690
CAC GAA GCA GCC AGC TCG TCC CAG CGT CGG CAG CCG CCC CCG GGC
His Glu Ala Ala Ser Ser Ser Gln Arg Arg Gln Pro Pro Gly Ser

          699         708         717         726         735
GCC GCG CAC CCG CTC AAC CGC AAG AGC CTT CTG GCC CCC GGA TCT
Pro Pro Gly Ala Ala His Pro Leu Asn Arg Lys Ser Leu Leu Ala

          744         753         762         771         780
GGC AGC GGC GGC GCG TCC CCA CTG ACC AGC GCG CAG GAC AGC GCC
Gly Ser Gly Gly Ala Ser Pro Leu Thr Ser Ala Gln Asp Ser Ala

          789         798         807         816         825
TTC CTC AAC GAC GCG GAC ATG GTC ATG AGC TTT GTG AAC CTG GTG
Phe Leu Asn Asp Ala Asp MET Val MET Ser Phe Val Asn Leu Val

          834         843         852         861         870
GAG TAC GAC AAG GAG TTC TCC CCT CGT CAG CGA CAC CAC AAA GAG
Glu Tyr Asp Lys Glu Phe Ser Pro Arg Gln Arg His His Lys Glu

          879         888         897         906         915
TTC AAG TTC AAC TTA TCC CAG ATT CCT GAG GGT GAG GTG GTG ACG
Phe Lys Phe Asn Leu Ser Gln Ile Pro Glu Gly Glu Val Val Thr

          924         933         942         951         960
GCT GCA GAA TTC CGC ATC TAC AAG GAC TGT GTT ATG GGG AGT TTT
Phe Arg Ile Tyr Lys Asp Cys Val MET Ala Ala Glu Gly Ser Phe
```

FIGURE 4C

```
          969          978          987          996         1005
AAA AAC CAA ACT TTT CTT ATC AGC ATT TAT CAA GTC TTA CAG GAG
Lys Asn Gln Thr Phe Leu Ile Ser Ile Tyr Gln Val Leu Gln Glu

         1014         1023         1032         1041         1050
CAT CAG CAC AGA GAC TCT GAC CTG TTT TTG TTG GAC ACC CGT GTA
His Gln His Arg Asp Ser Asp Leu Phe Leu Leu Asp Thr Arg Val

         1059         1068         1077         1086         1095
GTA TGG GCC TCA GAA GAA GGC TGG CTG GAA TTT GAC ATC ACG GCC
Val Trp Ala Ser Glu Glu Gly Trp Leu Glu Phe Asp Ile Thr Ala

         1104         1113         1122         1131         1140
ACT AGC AAT CTG TGG GTT GTG ACT CCA CAG CAT AAC ATG GGG CTT
Thr Ser Asn Leu Trp Val Val Thr Pro Gln His Asn MET Gly Leu

         1149         1158         1167         1176         1185
CAG CTG AGC GTG GTG ACA AGG GAT GGA GTC CAC GTC CAC CCC CGA
Gln Leu Ser Val Val Thr Arg Asp Gly Val His Val His Pro Arg

         1194         1203         1212         1221         1230
GCC GCA GGC CTG GTG GGC AGA GAC GGC CCT TAC GAT AAG CAG CCC
Ala Ala Gly Leu Val Gly Arg Asp Gly Pro Tyr Asp Lys Gln Pro

         1239         1248         1257         1266         1275
TTC ATG GTG GCT TTC TTC AAA GTG AGT GAG GTC CAC GTG CGC ACC
Phe MET Val Ala Phe Phe Lys Val Ser Glu Val His Val Arg Thr

         1284         1293         1302         1311         1320
ACC AGG TCA GCC TCC AGC CGG CGC CGA CAA CAG AGT CGT AAT CGC
Thr Arg Ser Ala Ser Ser Arg Arg Arg Gln Gln Ser Arg Asn Arg
                                                (382)

         1329         1338         1347         1356         1365
TCT ACC CAG TCC CAG GAC GTG GCG CGG GTC TCC AGT GCT TCA GAT
Ser Thr Gln Ser Gln Asp Val Ala Arg Val Ser Ser Ala Ser Asp
(388)

         1374         1383         1392         1401         1410
TAC AAC AGC AGT GAA TTG AAA ACA GCC TGC AGG AAG CAT GAG CTG
Tyr Asn Ser Ser Glu Leu Lys Thr Ala Cys Arg Lys His Glu Leu
                                                (412)

         1419         1428         1437         1446         1455
TAT GTG AGT TTC CAA GAC CTG GGA TGG CAG GAC TGG ATC ATT GCA
Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala
```

FIGURE 4D

```
      1464        1473        1482        1491        1500
CCC AAG GGC TAT GCT GCC AAT TAC TGT GAT GGA GAA TGC TCC TTC
Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu Cys Ser Phe

      1509        1518        1527        1536        1545
CCA CTC AAC GCA CAC ATG AAT GCA ACC AAC CAC GCG ATT GTG CAG
Pro Leu Asn Ala His MET Asn Ala Thr Asn His Ala Ile Val Gln

      1554        1563        1572        1581        1590
ACC TTG GTT CAC CTT ATG AAC CCC GAG TAT GTC CCC AAA CCG TGC
Thr Leu Val His Leu MET Asn Pro Glu Tyr Val Pro Lys Pro Cys

      1599        1608        1617        1626        1635
TGT GCG CCA ACT AAG CTA AAT GCC ATC TCG GTT CTT TAC TTT GAT
Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp

      1644        1653        1662        1671        1680
GAC AAC TCC AAT GTC ATT CTG AAA AAA TAC AGG AAT ATG GTT GTA
Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn MET Val Val

      1689        1698       1708       1718       1728
AGA GCT TGT GGA TGC CAC TAACTCGAAA CCAGATGCTG GGGACACACA
Arg Ala Cys Gly Cys His
                  (513)

     1738       1748       1758       1768       1778
TTCTGCCTTG GATTCCTAGA TTACATCTGC CTTAAAAAAA CACGGAAGCA

     1788       1798       1808       1818       1828
CAGTTGGAGG TGGGACGATG AGACTTTGAA ACTATCTCAT GCCAGTGCCT

     1838       1848       1858       1868       1878
TATTACCCAG GAAGATTTTA AAGGACCTCA TTAATAATTT GCTCACTTGG

     1888       1898       1908       1918       1928
TAAATGACGT GAGTAGTTGT TGGTCTGTAG CAAGCTGAGT TTGGATGTCT

     1938       1948       1958       1968       1978
GTAGCATAAG GTCTGGTAAC TGCAGAAACA TAACCGTGAA GCTCTTCCTA

     1988       1998       2008       2018       2028
CCCTCCTCCC CCAAAAACCC ACCAAAATTA GTTTTAGCTG TAGATCAAGC

     2038       2048       2058       2068       2078
TATTTGGGGT GTTTGTTAGT AAATAGGGAA AATAATCTCA AAGGAGTTAA

     2088       2098       2108       2118       2128
ATGTATTCTT GGCTAAAGGA TCAGCTGGTT CAGTACTGTC TATCAAAGGT
```

FIGURE 4E

```
      2138       2148       2158       2168       2178
AGATTTTACA GAGAACAGAA ATCGGGGAAG TGGGGGGAAC GCCTCTGTTC

      2188       2198       2208       2218       2228
AGTTCATTCC CAGAAGTCCA CAGGACGCAC AGCCCAGGCC ACAGCCAGGG

      2238       2248       2258       2268       2278
CTCCACGGGG CGCCCTTGTC TCAGTCATTG CTGTTGTATG TTCGTGCTGG

      2288       2298       2308       2318       2328
AGTTTTGTTG GTGTGAAAAT ACACTTATTT CAGCCAAAAC ATACCATTTC

      2338       2348       2358       2368       2378
TACACCTCAA TCCTCCATTT GCTGTACTCT TTGCTAGTAC CAAAAGTAGA

      2388       2398       2408       2418       2428
CTGATTACAC TGAGGTGAGG CTACAAGGGG TGTGTAACCG TGTAACACGT

      2438       2448       2458       2468       2478
GAAGGCAGTG CTCACCTCTT CTTTACCAGA ACGGTTCTTT GACCAGCACA

      2488       2498       2508       2518       2528
TTAACTTCTG GACTGCCGGC TCTAGTACCT TTTCAGTAAA GTGGTTCTCT

      2538       2548       2558       2568       2578
GCCTTTTTAC TATACAGCAT ACCACGCCAC AGGGTTAGAA CCAACGAAGA

      2588       2598       2608       2618       2628
AAATAAAATG AGGGTGCCCA GCTTATAAGA ATGGTGTTAG GGGGATGAGC

      2638       2648       2658       2668       2678
ATGCTGTTTA TGAACGGAAA TCATGATTTC CCTGTAGAAA GTGAGGCTCA

      2688       2698       2708       2718       2728
GATTAAATTT TAGAATATTT TCTAAATGTC TTTTTCACAA TCATGTGACT

      2738       2748       2758       2768       2778
GGGAAGGCAA TTTCATACTA AACTGATTAA ATAATACATT TATAATCTAC

      2788       2798       2808       2818       2828
AACTGTTTGC ACTTACAGCT TTTTTTGTAA ATATAAACTA TAATTTATTG

      2838       2848       2858       2868       2878
TCTATTTTAT ATCTGTTTTG CTGTGGCGTT GGGGGGGGGG CCGGGCTTTT

      2888       2898       2908       2918
GGGGGGGGGG GTTTGTTTGG GGGGTGTCGT GGTGTGGGCG GGCGG
```

FIGURE 5A

```
        10          20          30          40          50
CTGGTATATT TGTGCCTGCT GGAGGTGGAA TTAACAGTAA GAAGGAGAAA

        60          70          80          90          100
GGGATTGAAT GGACTTACAG GAAGGATTTC AAGTAAATTC AGGGAAACAC

        110         120         130         140         150
ATTTACTTGA ATAGTACAAC CTAGAGTATT ATTTTACACT AAGACGACAC

        160         170         180         190         200
AAAAGATGTT AAAGTTATCA CCAAGCTGCC GGACAGATAT ATATTCCAAC

        210         220         230         240         250
ACCAAGGTGC AGATCAGCAT AGATCTGTGA TTCAGAAATC AGGATTTGTT

        260         270         280         290         300
TTGGAAAGAG CTCAAGGGTT GAGAAGAACT CAAAAGCAAG TGAAGATTAC

        310         320         330         340         350
TTTGGGAACT ACAGTTTATC AGAAGATCAA CTTTTGCTAA TTCAAATACC

        360         370         380         390         400
AAAGGCCTGA TTATCATAAA TTCATATAGG AATGCATAGG TCATCTGATC

        410         420         430         440         450
AAATAATATT AGCCGTCTTC TGCTACATCA ATGCAGCAAA AACTCTTAAC

        460         470         480         490         500
AACTGTGGAT AATTGGAAAT CTGAGTTTCA GCTTTCTTAG AAATAACTAC

        510         520         530         540         550
TCTTGACATA TTCCAAAATA TTTAAAATAG GACAGGAAAA TCGGTGAGGA

        560         570         580         590         600
TGTTGTGCTC AGAAATGTCA CTGTCATGAA AAATAGGTAA ATTTGTTTTT

        610         620         630         640         650
TCAGCTACTG GGAAACTGTA CCTCCTAGAA CCTTAGGTTT TTTTTTTTTT

        660         670         680         690         700
AAGAGGACAA GAAGGACTAA AAATATCAAC TTTTGCTTTT GGACAAAA
```

FIGURE 5B

```
701          710          719          728          737
ATG CAT CTG ACT GTA TTT TTA CTT AAG GGT ATT GTG GGT TTC CTC
MET His Leu Thr Val Phe Leu Leu Lys Gly Ile Val Gly Phe Leu
(1)

746          755          764          773          782
TGG AGC TGC TGG GTT CTA GTG GGT TAT GCA AAA GGA GGT TTG GGA
Trp Ser Cys Trp Val Leu Val Gly Tyr Ala Lys Gly Gly Leu Gly

791          800          809          818          827
GAC AAT CAT GTT CAC TCC AGT TTT ATT TAT AGA AGA CTA CGG AAC
Asp Asn His Val  His Ser Ser Phe Ile Tyr Arg Arg Leu Arg Asn

836          845          854          863          872
CAC GAA AGA CGG GAA ATA CAA AGG GAA ATT CTC TCT ATC TTG GGT
His Glu Arg Arg Glu Ile Gln Arg Glu Ile Leu Ser Ile Leu Gly

881          890          899          908          917
TTG CCT CAC AGA CCC AGA CCA TTT TCA CCT GGA AAA ATG ACC AAT
Leu Pro His Arg Pro Arg Pro Phe Ser Pro Gly Lys Gln Ala Ser

926          935          944          953          962
CAA GCG TCC TCT GCA CCT CTC TTT ATG CTG GAT CTC TAC AAT GCC
Ser Ala Pro Leu Phe MET Leu Asp Leu Tyr Asn Ala MET Thr Asn

971          980          989          998         1007
GAA GAA AAT CCT GAA GAG TCG GAG TAC TCA GTA AGG GCA TCC TTG
Glu Glu Asn Pro Glu Glu Ser Glu Tyr Ser Val Arg Ala Ser Leu

1016        1025         1034         1043         1052
GCA GAA GAG ACC AGA GGG GCA AGA AAG GGA TAC CCA GCC TCT CCC
Ala Glu Glu Thr Arg Gly Ala Arg Lys Gly Tyr Pro Ala Ser Pro

1061        1070         1079         1088         1097
AAT GGG TAT CCT CGT CGC ATA CAG TTA TCT CGG ACG ACT CCT CTG
Asn Gly Tyr Pro Arg Arg Ile Gln Leu Ser Arg Thr Thr Pro Leu

1106        1115         1124         1133         1142
ACC ACC CAG AGT CCT CCT CTA GCC AGC CTC CAT GAT ACC AAC TTT
Thr Thr Gln Ser Pro Pro Leu Ala Ser Leu His Asp Thr Asn Phe

1151        1160         1169         1178         1187
CTG AAT GAT GCT GAC ATG GTC ATG AGC TTT GTC AAC TTA GTT GAA
Leu Asn Asp Ala Asp MET Val MET Ser Phe Val Asn Leu Val Glu

1196        1205         1214         1223         1232
AGA GAC AAG GAT TTT TCT CAC CAG CGA AGG CAT TAC AAA GAA TTT
Arg Asp Lys Asp Phe Ser His Gln Arg Arg His Tyr Lys Glu Phe
```

FIGURE 5C

```
1241        1250        1259        1268        1277
 CGA TTT GAT CTT ACC CAA ATT CCT CAT GGA GAG GCA GTG ACA GCA
 Arg Phe Asp Leu Thr Gln Ile Pro His Gly Glu Ala Val Thr Ala

1286        1295        1304        1313        1322
 GCT GAA TTC CGG ATA TAC AAG GAC CGG AGC AAC AAC CGA TTT GAA
 Ala Glu Phe Arg Ile Tyr Lys Asp Arg Ser Asn Asn Arg Phe Glu

1331        1340        1349        1358        1367
 AAT GAA ACA ATT AAG ATT AGC ATA TAT CAA ATC ATC AAG GAA TAC
 Asn Glu Thr Ile Lys Ile Ser Ile Tyr Gln Ile Ile Lys Glu Tyr

1376        1385        1394        1403        1412
 ACA AAT AGG GAT GCA GAT CTG TTC TTG TTA GAC ACA AGA AAG GCC
 Thr Asn Arg Asp Ala Asp Leu Phe Leu Leu Asp Thr Arg Lys Ala

1421        1430        1439        1448        1457
 CAA GCT TTA GAT GTG GGT TGG CTT GTC TTT GAT ATC ACT GTG ACC
 Gln Ala Leu Asp Val Gly Trp Leu Val Phe Asp Ile Thr Val Thr

1466        1475        1484        1493        1502
 AGC AAT CAT TGG GTG ATT AAT CCC CAG AAT AAT TTG GGC TTA CAG
 Ser Asn His Trp Val Ile Asn Pro Gln Asn Asn Leu Gly Leu Gln

1511        1520        1529        1538        1547
 CTC TGT GCA GAA ACA GGG GAT GGA CGC AGT ATC AAC GTA AAA TCT
 Leu Cys Ala Glu Thr Gly Asp Gly Arg Ser Ile Asn Val Lys Ser

1556        1565        1574        1583        1592
 GCT GGT CTT GTG GGA AGA CAG GGA CCT CAG TCA AAA CAA CCA TTC
 Ala Gly Leu Val Gly Arg Gln Gly Pro Gln Ser Lys Gln Pro Phe

1601        1610        1619        1628        1637
 ATG GTG GCC TTC TTC AAG GCG AGT GAG GTA CTT CTT CGA TCC GTG
 MET Val Ala Phe Phe Lys Ala Ser Glu Val Leu Leu Arg Ser Val

1646        1655        1664        1673        1682
 AGA GCA GCC AAC AAA CGA AAA AAT CAA AAC CGC AAT AAA TCC AGC
 Arg Ala Ala Asn Lys Arg Lys Asn Gln Asn Arg Asn Lys Ser Ser
                                                      (329)

1691        1700        1709        1718        1727
 TCT CAT CAG GAC TCC TCC AGA ATG TCC AGT GTT GGA GAT TAT AAC
 Ser His Gln Asp Ser Ser Arg MET Ser Ser Val Gly Asp Tyr Asn
 (337)
```

FIGURE 5D

```
1736        1745        1754        1763        1772
 ACA AGT GAG CAA AAA CAA GCC TGT AAG AAG CAC GAA CTC TAT GTG
 Thr Ser Glu Gln Lys Gln Ala Cys Lys Lys His Glu Leu Tyr Val
                                        (356)

1781        1790        1799        1808        1817
 AGC TTC CGG GAT CTG GGA TGG CAG GAC TGG ATT ATA GCA CCA GAA
 Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
   (362)

1826        1835        1844        1853        1862
 GGA TAC GCT GCA TTT TAT TGT GAT GGA GAA TGT TCT TTT CCA CTT
 Gly Tyr Ala Ala Phe Tyr Cys Asp Gly Glu Cys Ser Phe Pro Leu

1871        1880        1889        1898        1907
 AAC GCC CAT ATG AAT GCC ACC AAC CAC GCT ATA GTT CAG ACT CTG
 Asn Ala His MET Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu

1916        1925        1934        1943        1952
 GTT CAT CTG ATG TTT CCT GAC CAC GTA CCA AAG CCT TGT TGT GCT
 Val His Leu MET Phe Pro Asp His Val Pro Lys Pro Cys Cys Ala

1961        1970        1979        1988        1997
 CCA ACC AAA TTA AAT GCC ATC TCT GTT CTG TAC TTT GAT GAC AGC
 Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser

2006        2015        2024        2033        2042
 TCC AAT GTC ATT TTG AAA AAA TAT AGA AAT ATG GTA GTA CGC TCA
 Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn MET Val Val Arg Ser


2051        2060        2070        2080        2090        2100
 TGT GGC TGC CAC TAATATTAAA TAATATTGAT AATAACAAAA AGATCTGTAT
 Cys Gly Cys His
           (454)
      2110        2120        2130        2140        2150
TAAGGTTTAT GGCTGCAATA AAAAGCATAC TTTCAGACAA ACAGAAAAAA AAA
```

Figure 6

```
          (1)
GAATTCC GAG CCC CAT TGG AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT
        Glu Pro His Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala
                                          (10)

GGG GAG GCG GTC ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC
Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His
          (20)                                    (30)

CTG CTC AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC
Leu Leu Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser
                    (40)                                    (50)

AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT GGA GAC
Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp
                          (60)                                    (70)

GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC TGG TTG CTG AAG
Glu Gly Typ Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cyc Trp Leu Leu Lys
                                (80)

CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG ACT GAG GAT GGG CAC AGC
Arg His Lys Asp Leu Gly Lue Arg Leu Tyr Val Glu Thr Glu Asp Gly His Ser
    (90)                                (100)

GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT CAA CGG GCC CCA CGC TCC CAA CAG
Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser Gln Gln
          (110)                                    (120)

CCT TTC GTG GTC ACT TTC TTC AGG GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG
Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg
                    (130)                                    (140)

GCA GTG AGG CCA CTG AGG AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG
Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln
                          (150)                                    (160)

GCC AAC CGA CTC CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG
Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln
                                (170)

GTC TGC CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTT GGC TGG CTG GAC
Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp
   (180)                                (190)

TGG GTC ATC GCC CCC CAA GGC TAC TCA GCC TAT TAC TGT GAG GGG GAG TGC TCC
Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser
          (200)                                    (210)

TTC CCG CTG GAC TCC TGC ATG AAC GCC ACC AAC CAC GCC ATC CTG CAG TCC CTG
Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu
                    (220)                                    (230)
```

Figure 6 (Con't)

```
GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG TGC TGT GCA CCC ACC AAG
Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys
                          (240)                                      (250)

CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC AGC AGC AAC AAC GTC ATC CTG CGC
Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg
                                    (260)

AAG CAC CGC AAC ATG GTG GTC AAG GCC TGC GGC TGC CAC TGAGTCAGCCCGCCCAGC
Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
   (270)                                      (280)

CCTACTGCAGCCACCCTTCTCATCTGGATCGGGCCCTGCAGAGGCAGAAAACCCTTAAATGCTGTCACAG

CTCAAGCAGGAGTGTCAGGGGCCCTCACTCTCGGTGCCTACTTCCTGTCAGGCTTCTGGGAATTC
```

FIGURE 7

GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT 60
CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT 120
TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT 180
AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT 240
TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG 300
CTGAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA 360
TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC 420
TATGTGGCGC GGTATTATCC CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC 480
ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG 520
GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA 600
ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG 660
GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT GAATGAAGCC ATACCAAACG 720
ACGAGCGTGA CACCACGATG CCTGTAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACTG 780
GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG 840
TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG 900
GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT 960
CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC 1020
AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT 1080
CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA 1140
TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT 1200
CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTTCTG CGCGTAATCT 1260
GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC 1320
TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC 1380
TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC 1440
TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG 1500
GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT 1560
CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG 1620
AGCATTGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG 1680
GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT 1740
ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTTGTGA TGCTCGTCAG 1800
GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT 1860
GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA 1920

FIGURE 7 (cont'd)

TTACCGCCTT TCAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT 1980
CAGTGAGCGA GGAAGCGGAA GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC 2040
CGATTCATTA ATGCAGAATT GATCTCTCAC CTACCAAACA ATGCCCCCCT GCAAAAAATA 2100
AATTCATATA AAAAACATAC AGATAACCAT CTGCGGTGAT AAATTATCTC TGGCGGTGTT 2160
GACATAAATA CCACTGGCGG TGATACTGAG CACATCAGCA GGACGCACTG ACCACCATGA 2220
AGGTGACGCT CTTAAAAATT AAGCCCTGAA GAAGGGCAGC ATTCAAAGCA GAAGGCTTTG 2280
GGGTGTCTGA TACGAAACGA AGCATTGGCC GTAAGTGCGA TTCCGGATTA GCTGCGAATG 2340
TGCCAATCGC GGGGGGTTTT CGTTCAGGAC TACAACTGCC ACACACCACC AAAGCTAACT 2400
GACAGGAGAA TCCAGATGGA TGCACAAACA CGCCGCCGCG AACGTCGCGC AGAGAAACAG 2460
GCTCAATGGA AAGCAGCAAA TCCCCTGTTG GTTGGGGTAA GCGCAAAACC AGTTCCGAAA 2520
GATTTTTTTA ACTATAAACG CTGATGGAAG CGTTTATGCG GAAGAGGTAA AGCCCTTCCC 2580
CAGTAACAAA AAAACAACAG CATAAATAAC CCCGCTCTTA CACATTCCAG CCCTGAAAAA 2640
GGGCATCAAA TTAAACCACA CCTATGGTGT ATGCATTTAT TTGCATACAT TCAATCAATT 2700
GTTATCTAAG GAAATACTTA CATATGCAAG CTAAACATAA ACAACGTAAA CGTCTGAAAT 2760
CTAGCTGTAA GAGACACCCT TTGTACGTGG ACTTCAGTGA CGTGGGGTGG AATGACTGGA 2820
TTGTGGCTCC CCCGGGGTAT CACGCCTTTT ACTGCCACGG AGAATGCCCT TTTCCTCTGG 2880
CTGATCATCT GAACTCCACT AATCATGCCA TTGTTCAGAC GTTGGTCAAC TCTGTTAACT 2940
CTAAGATTCC TAAGGCATGC TGTGTCCGGA CAGAACTCAG TGCTATCTCG ATGCTGTACC 3000
TTGACGAGAA TGAAAAGGTT GTATTAAAGA ACTATCAGGA CATGGTTGTG GAGGGTTGTG 3060
GGTGTCGCTA GTACAGCAAA ATTAAATACA TAAATATATA TATATATATA TATTTTAGAA 3120
AAAAGAAAAA AATCTAGAGT CGACCTGCAG TAATCGTACA GGGTAGTACA AATAAAAAAG 3180
GCAGGTCAGA TGACGTGCCT TTTTTCTTGT GAGCAGTAAG CTTGGCACTG GCCGTCGTTT 3240
TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC 3300
CCCCTTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT 3360
TGCGCAGCCT GAATGGCGAA TGGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG 3420
GTATTTCACA CCGCATATAT GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT 3480
AAGCCAGCCC CGACACCCGC CAACACCCGC TGACGCGCCC TGACGGGCTT GTCTGCTCCC 3540
GGCATCCGCT TACAGACAAG CTGTGACCGT CTCCGGGAGC TGCATGTGTC AGAGGTTTTC 3600
ACCGTCATCA CCGAAACGCG CGA 3623

FIGURE 8

W-20 ALKALINE PHOSPHATASE: BMP-2 VS. BMP-2/7

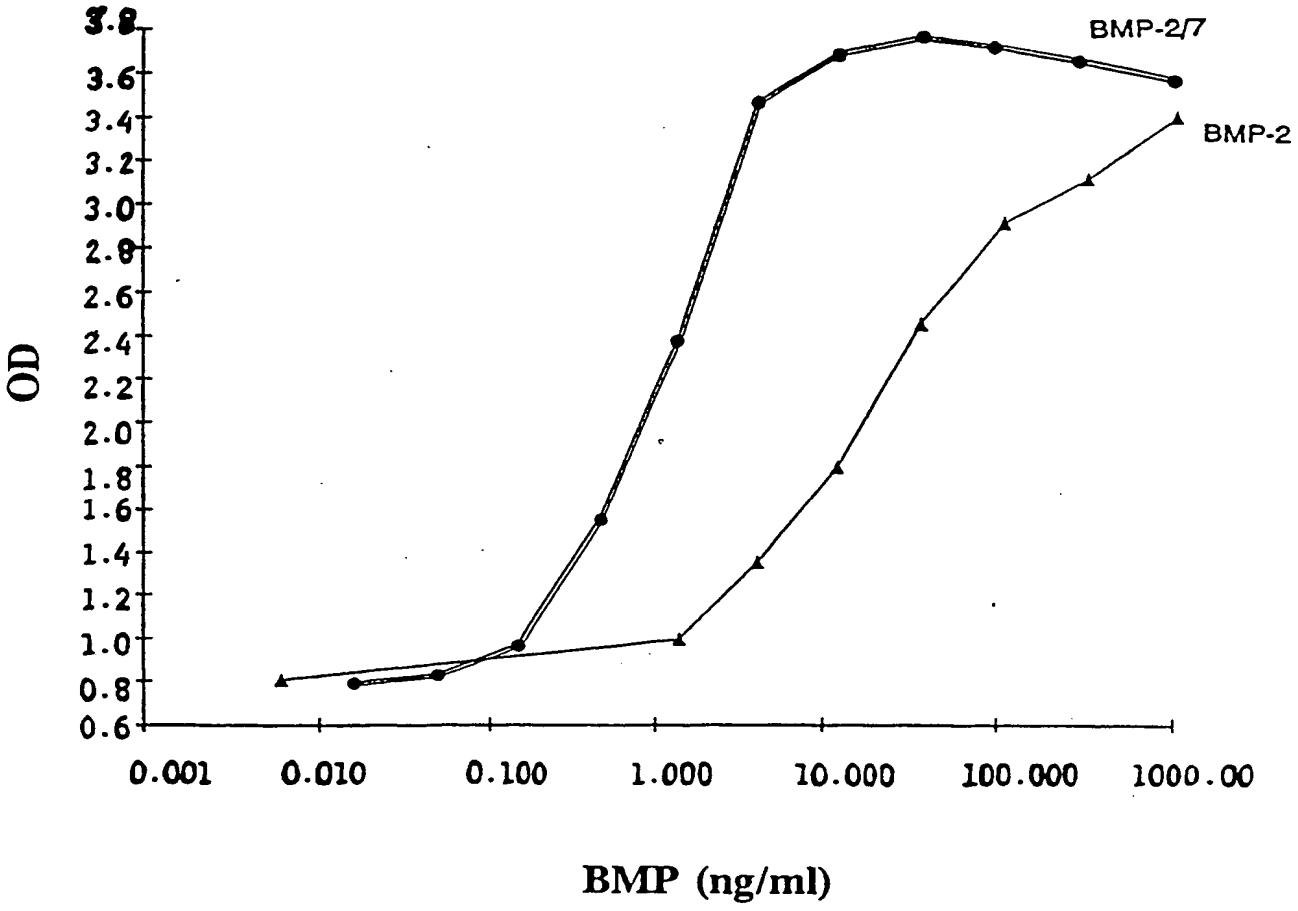


FIGURE 9

EFFECTS OF BMP-2 AND BMP2/7 ON BGP SYNTHESIS BY W-20 CELLS

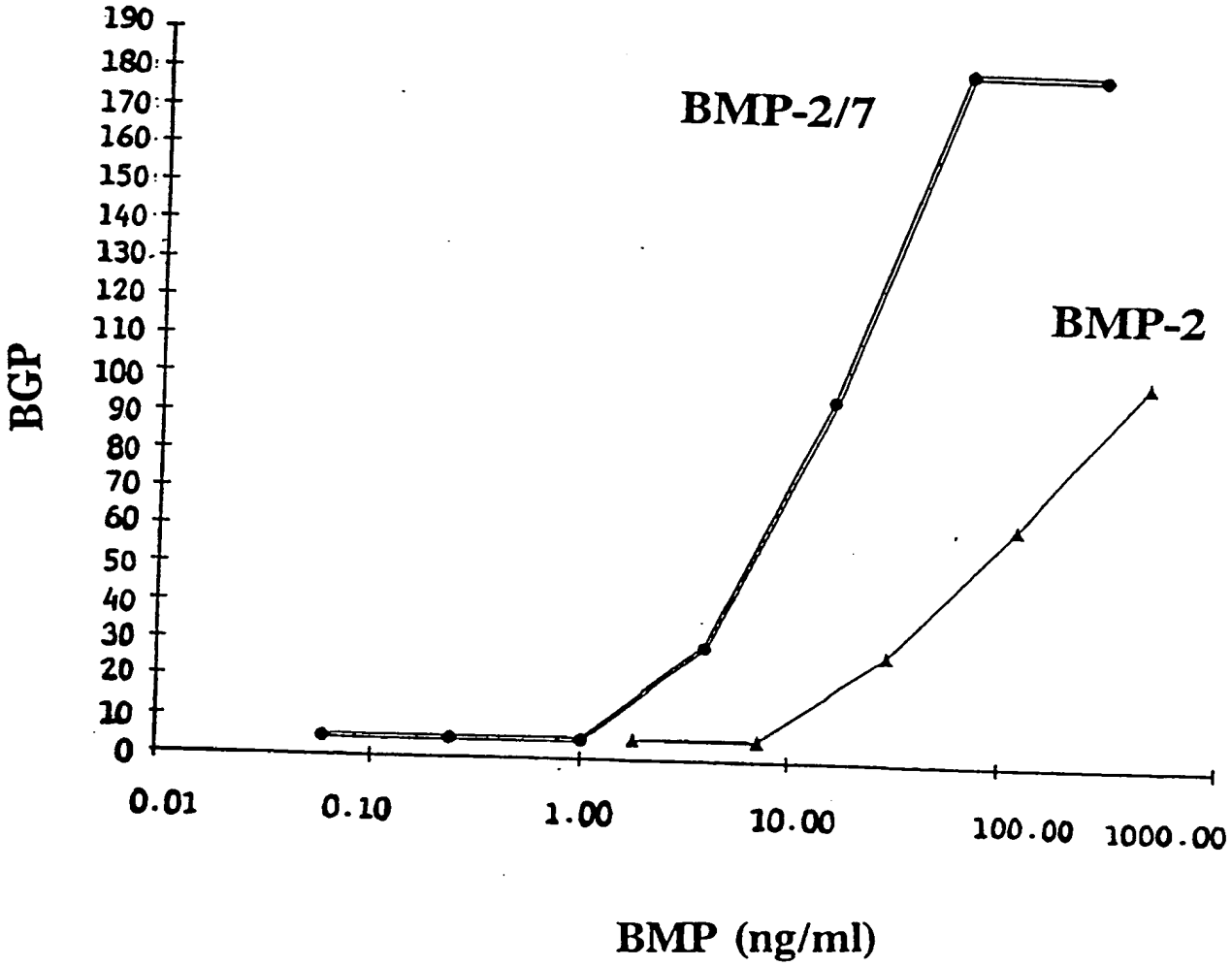


FIGURE 10

COMPARAISON OF *E.Coli* BMP-2 AND BMP-2/7: W-20-17 ALKALINE PHOSPHATASE

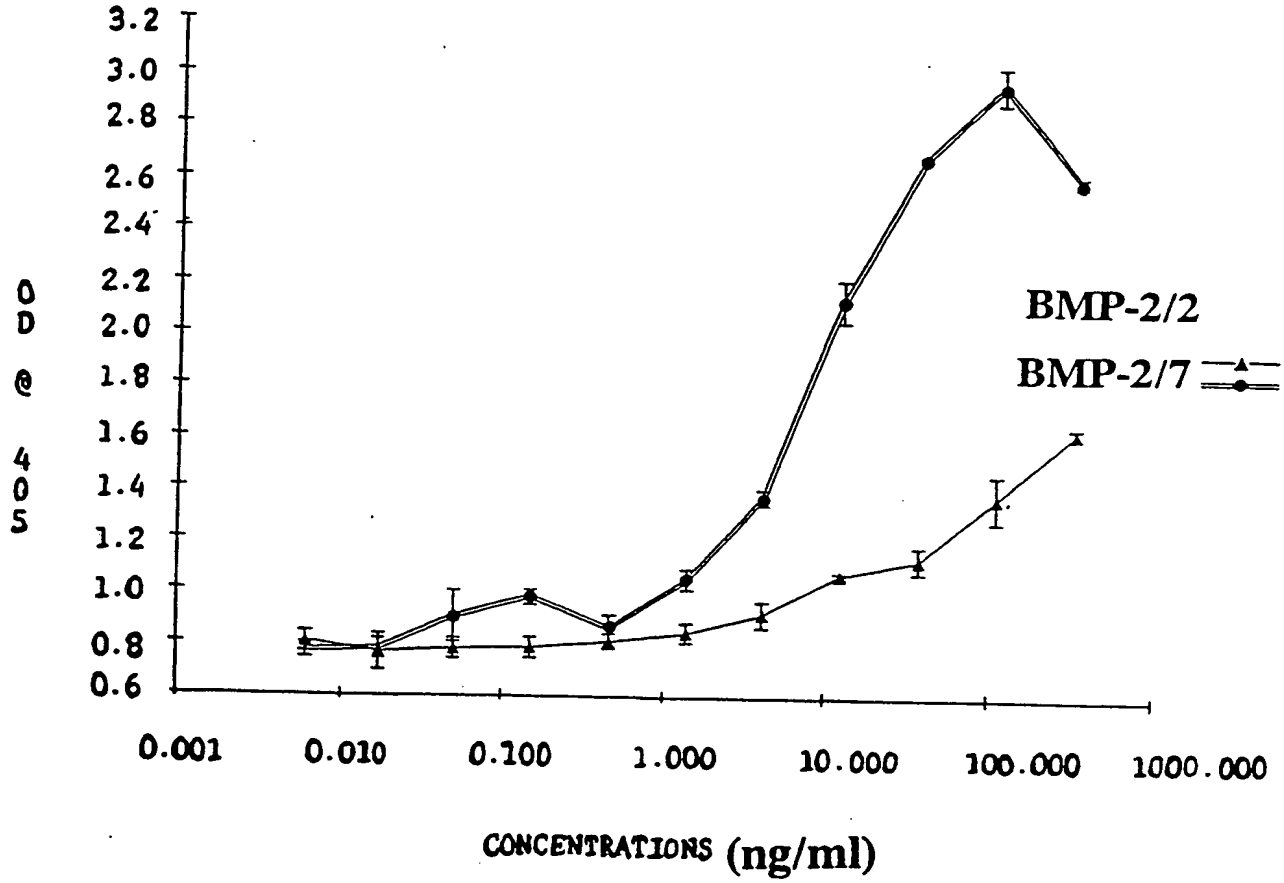


FIGURE 11A

```
        10         20         30         40         50         60         70
AGATCTTGAA AACACCCGGG CCACACACGC CGCGACCTAC AGCTCTTTCT CAGCGTTGGA GTGGAGACGG

        80         90        100        110        120        130        140
CGCCCGCAGC GCCCTGCGCG GGTGAGGTCC GCGCAGCTGC TGGGGAAGAG CCCACCTGTC AGGCTGCGCT

       150        160        170        180        190        200        210
GGGTCAGCGC AGCAAGTGGG GCTGGCCGCT ATCTCGCTGC ACCCGGCCGC GTCCCGGGCT CCGTGCGCCC

       220        230        240        250        260        270        280
TCGCCCCAGC TGGTTTGGAG TTCAACCCTC GGCTCCGCCG CCGGCTCCTT GCGCCTTCGG AGTGTCCCGC

       290        300        310        320 (1)            335
AGCGACGCCG GGAGCCGACG CGCCGCGCGG GTACCTAGCC ATG GCT GGG GCG AGC AGG CTG CTC
                                           MET Ala Gly Ala Ser Arg Leu Leu

    350                365                380                395
TTT CTG TGG CTG GGC TGC TTC TGC GTG AGC CTG GCG CAG GGA GAG AGA CCG AAG CCA
Phe Leu Trp Leu Gly Cys Phe Cys Val Ser Leu Ala Gln Gly Glu Arg Pro Lys Pro

        410                425                440                455
CCT TTC CCG GAG CTC CGC AAA GCT GTG CCA GGT GAC CGC ACG GCA GGT GGT GGC CCG
Pro Phe Pro Glu Leu Arg Lys Ala Val Pro Gly Asp Arg Thr Ala Gly Gly Gly Pro

            470                485                500                515
GAC TCC GAG CTG CAG CCG CAA GAC AAG GTC TCT GAA CAC ATG CTG CGG CTC TAT GAC
Asp Ser Glu Leu Gln Pro Gln Asp Lys Val Ser Glu His MET Leu Arg Leu Tyr Asp

                530                545                560
AGG TAC AGC ACG GTC CAG GCG GCC CGG ACA CCG GGC TCC CTG GAG GGA GGC TCG CAG
Arg Tyr Ser Thr Val Gln Ala Ala Arg Thr Pro Gly Ser Leu Glu Gly Gly Ser Gln

575                590                605                620
CCC TGG CGC CCT CGG CTC CTG CGC GAA GGC AAC ACG GTT CGC AGC TTT CGG GCG GCA
Pro Trp Arg Pro Arg Leu Leu Arg Glu Gly Asn Thr Val Arg Ser Phe Arg Ala Ala

    635                650                665                680
GCA GCA GAA ACT CTT GAA AGA AAA GGA CTG TAT ATC TTC AAT CTG ACA TCG CTA ACC
Ala Ala Glu Thr Leu Glu Arg Lys Gly Leu Tyr Ile Phe Asn Leu Thr Ser Leu Thr

        695                710                725                740
AAG TCT GAA AAC ATT TTG TCT GCC ACA CTG TAT TTC TGT ATT GGA GAG CTA GGA AAC
Lys Ser Glu Asn Ile Leu Ser Ala Thr Leu Tyr Phe Cys Ile Gly Glu Leu Gly Asn
```

FIGURE 11C

```
1430                1445    (377)       1460                1475
TGC GCC AGG AGA TAC CTC AAG GTA GAC TTT GCA GAT ATT GGC TGG AGT GAA TGG ATT
Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser Glu Trp Ile

   1490                1505                1520                1535
ATC TCC CCC AAG TCC TTT GAT GCC TAT TAT TGC TCT GGA GCA TGC CAG TTC CCC ATG
Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly Ala Cys Gln Phe Pro MET

        1550                1565                1580                1595
CCA AAG TCT TTG AAG CCA TCA AAT CAT GCT ACC ATC CAG AGT ATA GTG AGA GCT GTG
Pro Lys Ser Leu Lys Pro Ser Asn His Ala Thr Ile Gln Ser Ile Val Arg Ala Val

            1610                1625                1640                1655
GGG GTC GTT CCT GGG ATT CCT GAG CCT TGC TGT GTA CCA GAA AAG ATG TCC TCA CTC
Gly Val Val Pro Gly Ile Pro Glu Pro Cys Cys Val Pro Glu Lys MET Ser Ser Leu

                1670                1685                1700
AGT ATT TTA TTC TTT GAT GAA AAT AAG AAT GTA GTG CTT AAA GTA TAC CCT AAC ATG
Ser Ile Leu Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn MET

1715                1730    (472)       1746        1756        1766        1776
ACA GTA GAG TCT TGC GCT TGC AGA TAACCTGGCA AAGAACTCAT TTGAATGCTT AATTCAATCT
Thr Val Glu Ser Cys Ala Cys Arg

      1786
CTAGAGTCGA CGGAATTC
```

Figure 12

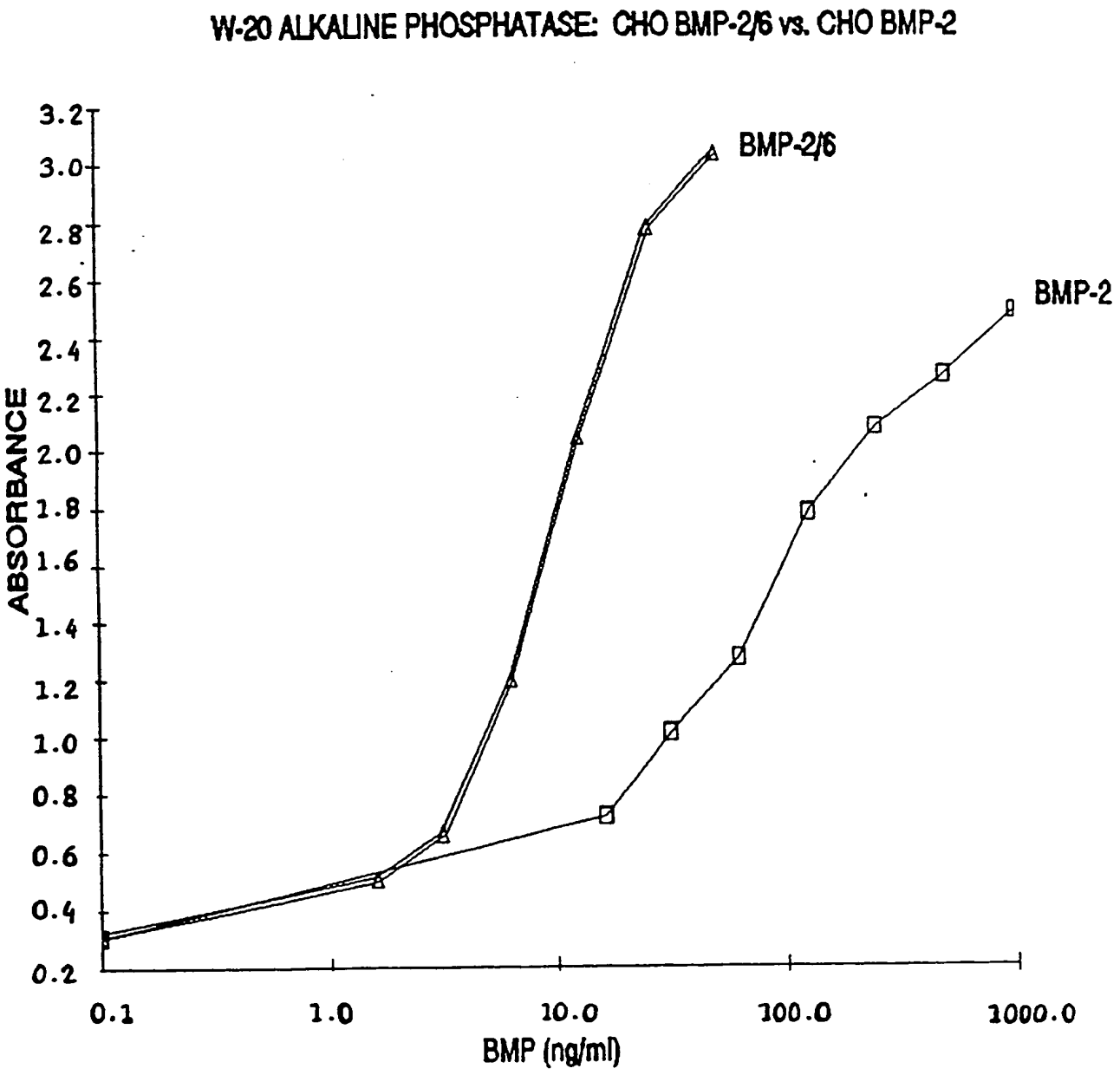


FIGURE 13A

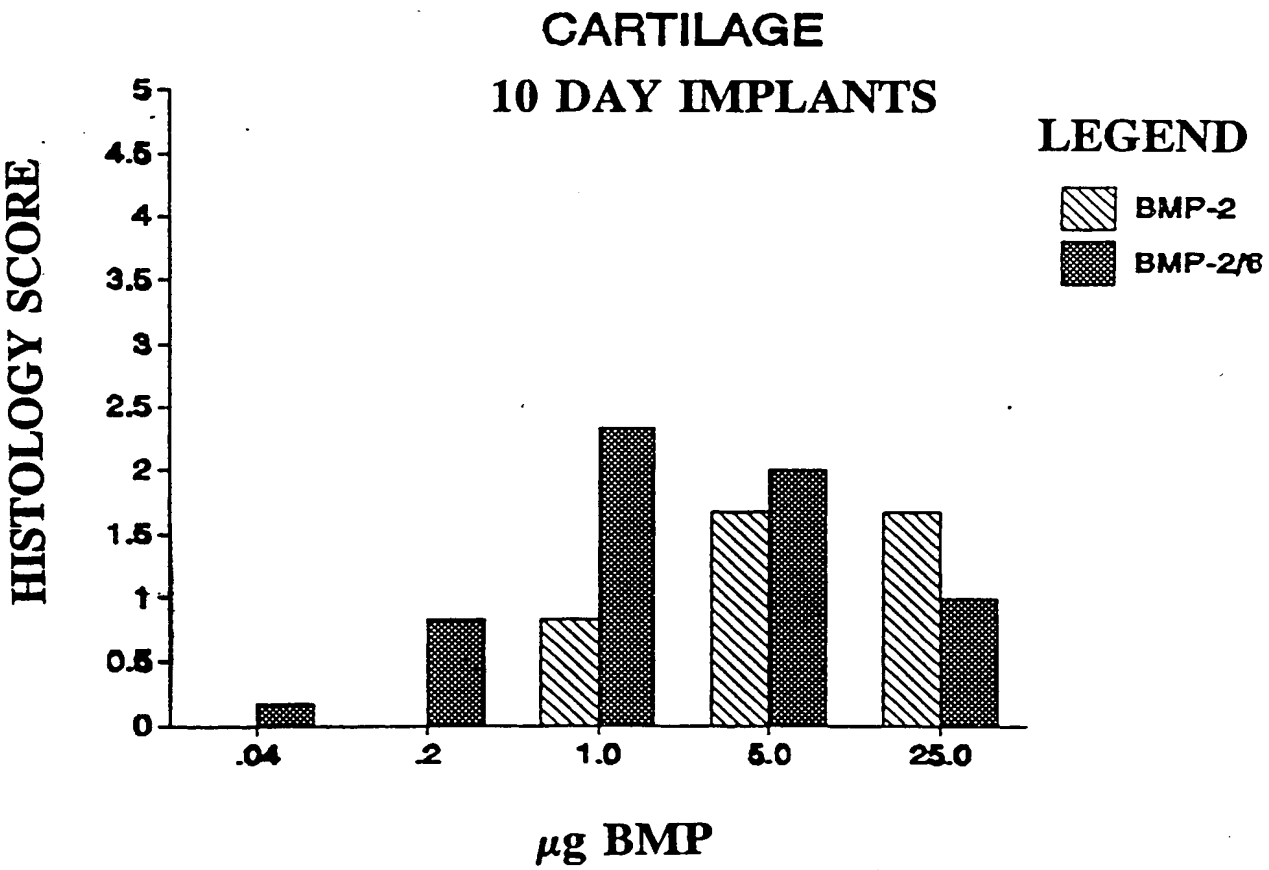


FIGURE 13B

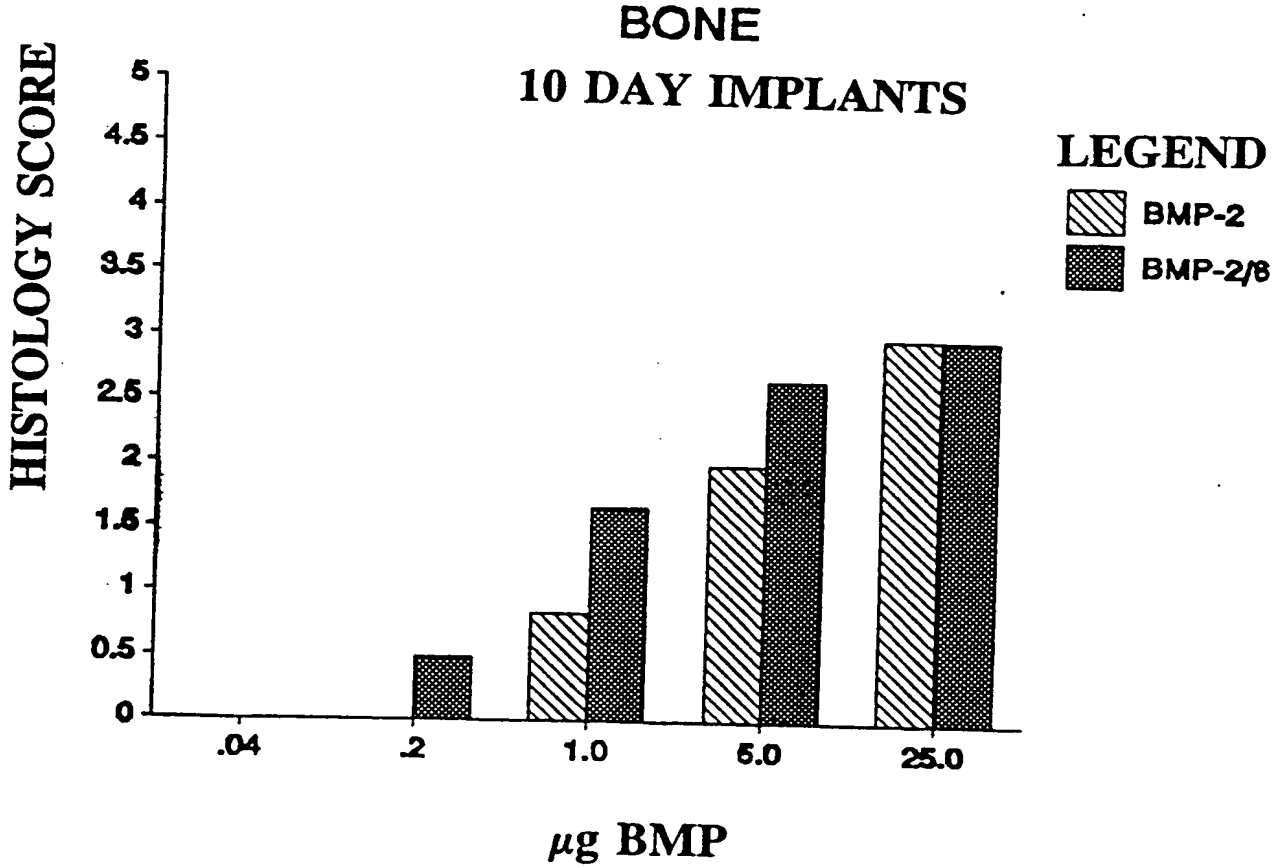


FIGURE 14A

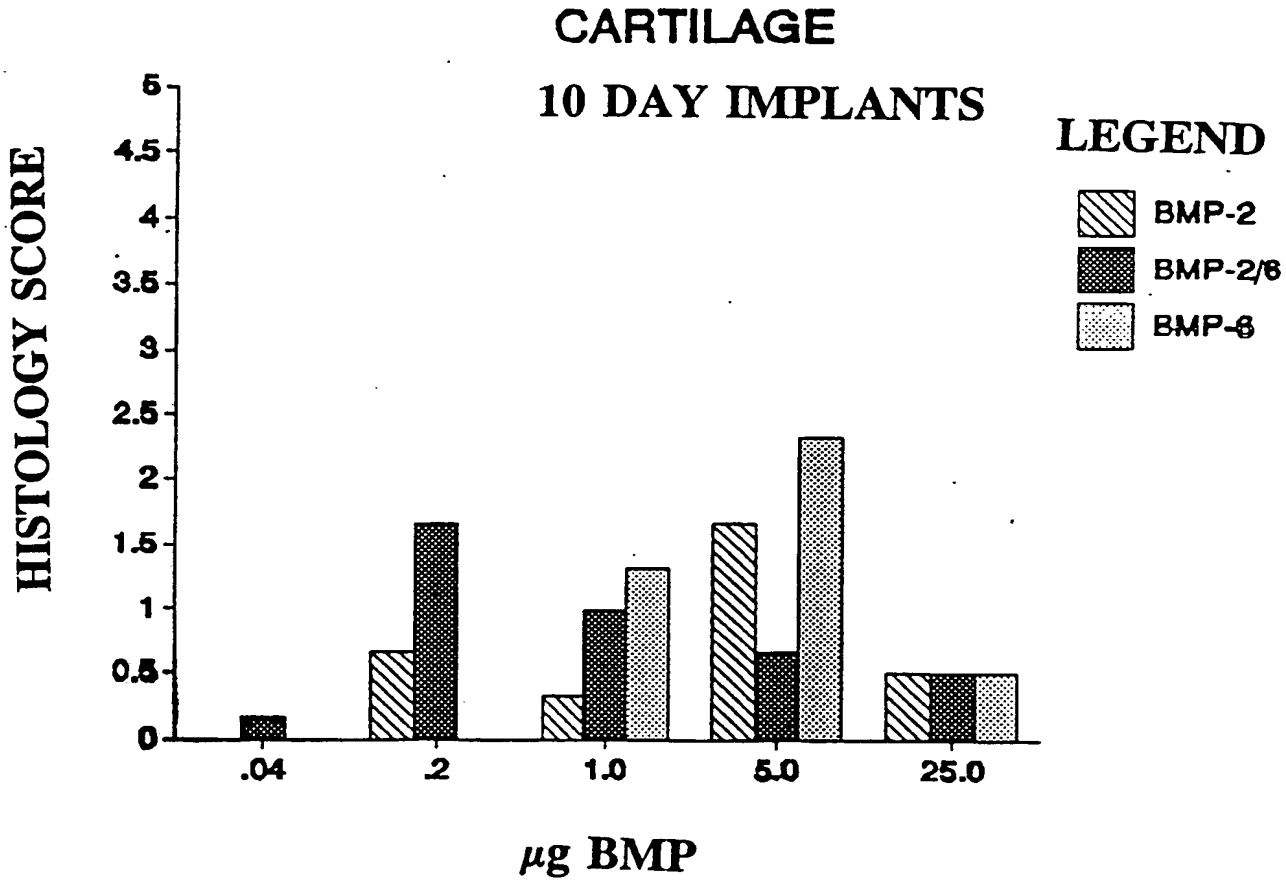
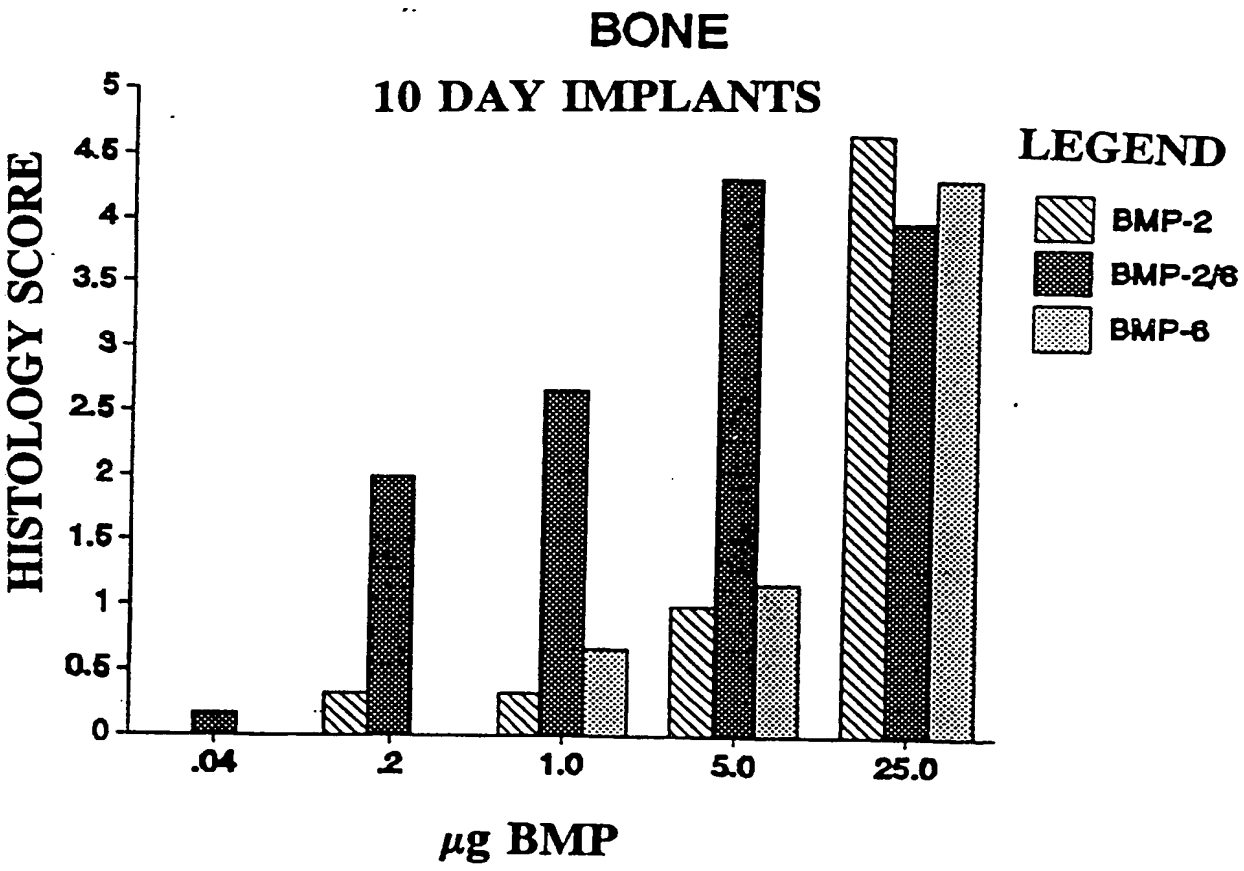


FIGURE 14B



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/09430

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/12; C12P21/02; A61K37/02; C12N5/12
C07K15/06

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; C12N ; A61K ; C12P

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	WO,A,9 003 733 (INTERNATIONAL GENETIC ENGINEERING, INC.) 19 April 1990 see page 16, line 7 - page 17, line 28 see page 18, line 22 - line 34	1,4, 7-14,16, 23-26
Y	see page 51, line 32 - page 52, line 10; figure 12 see page 62 - page 63; claim 35	13-17, 33,35

Y	WO,A,9 011 366 (GENETICS INSTITUTE, INC.) 4 October 1990 cited in the application see page 22, line 20 - line 27 see page 43, line 17 - line 30	13-16,33

	-/--	

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
04 FEBRUARY 1993	26. 02. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	ANDRES S.M.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO,A,8 910 409 (GENETICS INSTITUTE, INC.) 2 November 1989 cited in the application see page 7, line 13 - line 15 see page 8, line 20 - line 29	1,4, 7-12,23, 25-26
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 87, March 1990, WASHINGTON US pages 2220 - 2224 WANG, E.A. ET AL. 'Recombinant human bone morphogenetic protein induces bone formation' cited in the application	34,36
Y	see figure 1C	35
X	JOURNAL OF BIOLOGICAL CHEMISTRY vol. 265, no. 22, 5 August 1990, BALTIMORE, MD US pages 13198 - 13205 SAMPATH, T.K. ET AL. 'Bovine osteogenic protein is composed of dimers of OP-1 and BMP-2A, two members of the transforming growth factor-beta superfamily' see the whole document	34,36
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 87, no. 24, December 1990, WASHINGTON US pages 9843 - 9847 CELESTE, A.J. ET AL. 'Identification of transforming growth factor-beta family members present in bone-inductive protein purified from bovine bone'	34,36
A	see page 9846, left column, line 13 - right column, line 7 see page 9847, left column, paragraph 2-3	13,16
A	WO,A,8 909 787 (CREATIVE BIOMOLECULES, INC.) 19 October 1989 see page 6, line 22 - line 24 see page 56, paragraphs E5 & E6	
P,Y	WO,A,9 118 098 (GENETICS INSTITUTE, INC.) 28 November 1991 cited in the application see page 12, line 31 - page 13, line 7	17

-/--

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	JOURNAL OF CELLULAR BIOCHEMISTRY Supplement 16F, 1992, page 76, abstract W026; WOZNEY, J.M. ET AL.: 'Regulation of chondrogenesis and osteogenesis by the BMP proteins' see abstract & Keystone Symposium on growth and differentiation factors in vertebrate development; Keystone, Colorado, USA April 3-16, 1992 -----	1

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9209430
SA 66918

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 04/02/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9003733	19-04-90	US-A-	5106626	21-04-92
		AU-B-	615810	10-10-91
		AU-A-	4488689	01-05-90
		CA-A-	2000498	11-04-90
		EP-A-	0394418	31-10-90
		JP-T-	4505151	10-09-92
WO-A-9011366	04-10-90	US-A-	5106748	21-04-92
		US-A-	5141905	25-08-92
		AU-A-	5357790	22-10-90
		CA-A-	2030518	29-09-90
		EP-A-	0429570	05-06-91
		JP-T-	3505098	07-11-91
WO-A-8910409	02-11-89	AU-A-	3448789	24-11-89
		EP-A-	0408649	23-01-91
		JP-T-	3503649	15-08-91
		US-A-	5106748	21-04-92
WO-A-8909787	19-10-89	US-A-	4968590	06-11-90
		US-A-	5011691	30-04-91
		AU-B-	628050	10-09-92
		AU-A-	3444989	03-11-89
		AU-B-	618357	19-12-91
		AU-A-	3530589	03-11-89
		EP-A-	0372031	13-06-90
		EP-A-	0362367	11-04-90
		JP-T-	3500655	14-02-91
		JP-T-	3502579	13-06-91
		WO-A-	8909788	19-10-89
		US-A-	5108753	28-04-92
		AU-B-	627850	03-09-92
		AU-A-	5174790	26-09-90
		EP-A-	0411105	06-02-91
		JP-T-	3504736	17-10-91
		WO-A-	9010018	07-09-90
		US-A-	4975526	04-12-90
		US-A-	5171574	15-12-92
		US-A-	5162114	10-11-92

EPO FORM P0479

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9209430
SA 66918

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

04/02/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9118098	28-11-91	None	

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82